

EXPERIMENTAL PHYSIOLOGY AND ANATOMY

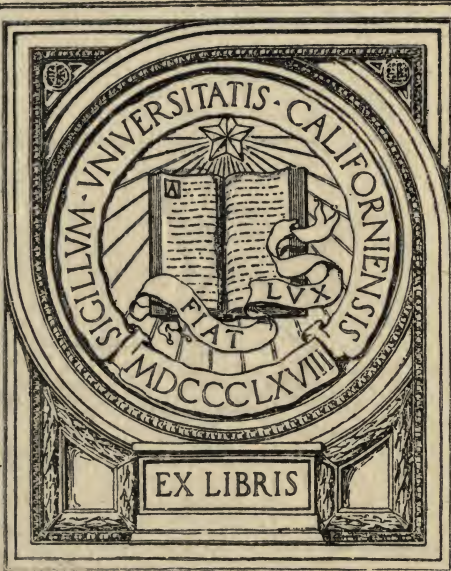
WALTER HOLLIS EDDY

UC-NRLF



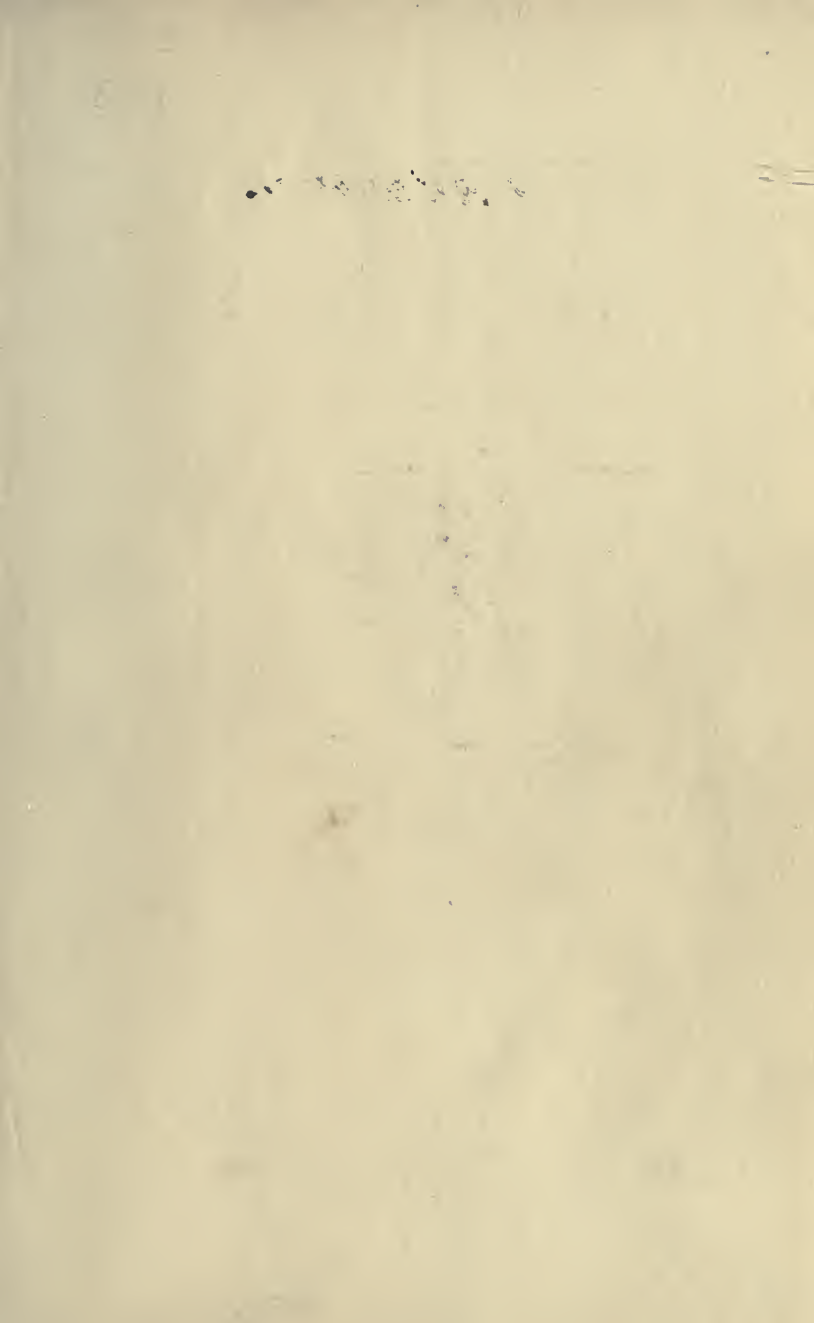
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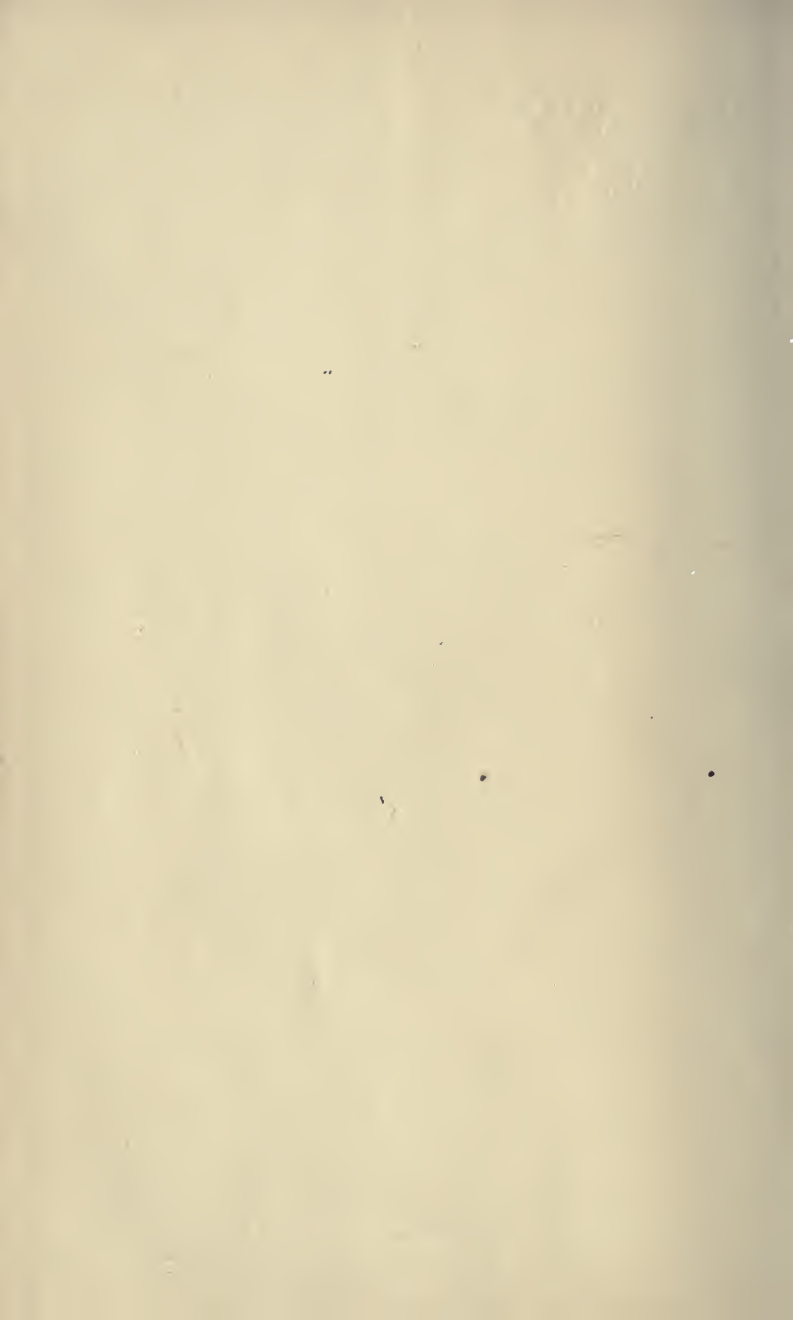
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EXPERIMENTAL PHYSIOLOGY AND ANATOMY

FOR
HIGH SCHOOLS

BY
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Eddy's Experimental Physiology

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PREFACE

THOUGH the importance of Physiology in secondary schools is everywhere recognized, little attempt has been made to place the subject on an experimental basis. This book has been prepared in an effort to call attention to the great field which this subject presents for laboratory study.

The starred topics in the following table of contents constitute a brief course covering that which is essential; and the optional exercises make it possible to extend the work at the discretion of the teacher.¹ The ingenuity of the teacher will readily suggest substitutes for the material suggested when the laboratory facilities of the school are inadequate.

Some of the exercises may be made demonstrations, and time in school may also be saved by assigning some of the simpler exercises as part of the home work of the pupil.

I wish to acknowledge the many helpful suggestions given me by my colleagues of the High School of Commerce and by members of the Columbia University faculty. I have also found many useful suggestions in the works of Messrs. Foster and Langley, J. E. Peabody, M. L. Macy, H. Newell Martin, Hammarsten, Verworn, Wilson, and Schäfer.

I wish also to express my great indebtedness to Dr. E. A. Darling of Harvard College and to Mr. Frank O. Payne of the High School of Commerce for their critical review of the manuscript and for the aid they have given me in its preparation; and to my wife for great assistance in the many details of grammatical arrangement and mechanical labor involved in the work.

W. H. E.

THE HIGH SCHOOL OF COMMERCE,
NEW YORK CITY.

¹ The book in its starred topics meets the requirements of the New York State Syllabus, and as a whole has been accepted by the Harvard College authorities as meeting the entrance requirements of that institution.

METHOD OF EXPERIMENT

It has been the purpose of the author so to state each of the following exercises as to admit of its performance by the pupil with a minimum amount of direction from the teacher. Most of the exercises should be thus performed by each pupil individually, or by two pupils together; but of course the teacher may select as many as desired for performance as demonstrations before the class.

It is essential that each pupil make a suitable record of all exercises performed, in a carefully prepared notebook. It is recommended that a separate-leaf notebook be used for this purpose, as this makes possible the inspection of one set of exercises without handling the entire books, and permits the rewriting of unsatisfactory work without disturbing the arrangement of the book.

It is generally agreed, too, that the book should consist of original reports made at the time of experiment, and not of matter copied from original rough drafts.

Frequent examination of all laboratory notes by the teacher is also essential to good work, and the proper status of the notebook work can be secured only by giving it a definite proportion in the marking of the pupil's work. A rubber stamp with the word "Approved" and the instructor's name may be obtained of any stationer at small expense and will facilitate the work of correction greatly. Neatness as well as accuracy and adequacy of report

should receive proper weight in the marking of notebook work.

When the work is completed the student should prepare an index of drawings, records of experiments, and descriptions of demonstrations contained in the notebook. It is well to indicate in this index, after each title, whether the work was done by the pupil or observed and recorded by him, and whether in the laboratory or as home work.

The following directions may prove of value as indicating a satisfactory method of arrangement of a notebook record:

- A. Record the number and date of the exercise.
- B. Make drawings of the apparatus used, when necessary, and label them properly.
- C. State as briefly as possible:
 - (1) What was done.
 - (2) What happened as the results.
 - (3) What meaning these results have, and the purpose of the exercise.
- D. Answer all questions in the text and try to condense your statements into as concise and brief form as possible.

The exercises as a rule should precede the text study and serve as a basis for such study.

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EXPERIMENTAL PHYSIOLOGY AND ANATOMY

PRELIMINARY EXERCISES

I.—GLASS BENDING AND CUTTING (OPTIONAL).

Apparatus.—Several pieces of quarter inch glass tubing about two feet in length, a three-cornered file, a Bunsen burner with fish-tail attachment.

Directions.—*A. Bending.* Place the fish-tail attachment on the burner and light the gas. Hold at the ends the tube which is to be bent and bring into the flame the

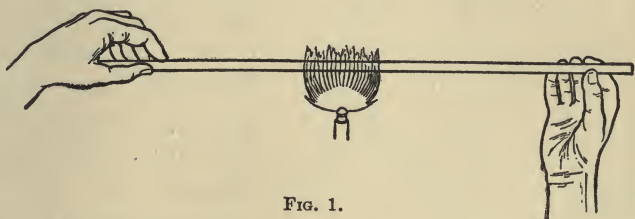


FIG. 1.

part at which you wish the bend (see Fig. 1). Turn the tube constantly to insure equal heating of all parts, and when the glass is flexible remove from the flame and bend the two ends slowly toward one another until the desired angle is obtained. Use care to keep the two ends in the same plane, and do not bend quickly, as that would cause buck-

ling. If the glass cools too soon return it to the flame and treat as before.

B. Cutting. Wet the file and, holding the tube firmly with finger and thumb, make a slight scratch across it. Turn the tube over and repeat the operation at a point directly opposite. Now grasp the tube in both hands, one on each side of the scratches, and bend sharply. The result should be a clean, square-ended break. The edges may be rounded by holding them in the flame a moment.

II.—COLLECTION OF GASES (OPTIONAL).

Apparatus.—Pneumatic trough and support, glass tube bent at right angles, large-mouthed bottles, piece of glass to cover mouth of bottle.

Directions.—*A.* Fill the trough with water to the depth of a half inch above the top of the support. Fill the bottle with water, cover the mouth with the glass, and invert, putting the mouth under the water of the trough. Remove the piece of glass, and place the bottle over one of the holes of the support. Does the water flow out? Explain.

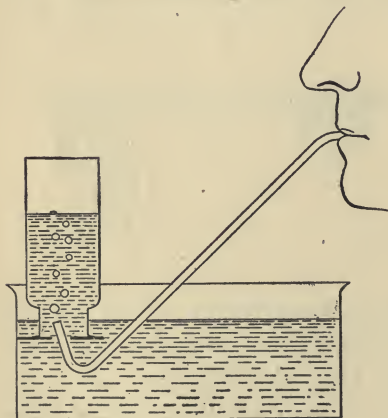


FIG. 2.

Now introduce the short end of the glass tube into the mouth of the bottle (see Fig. 2) and blow

through the other end. Where does this gas go? Why? Would this method of collecting gases be successful if they were readily soluble in water?

B. Fill a second bottle with water and invert in the same way as the first. Bring the one containing the gas under the one containing the water, and gradually turn it mouth upward (see Fig. 3). In this way gases may be transferred from one vessel to another for study.

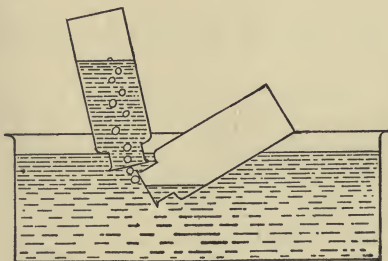


FIG. 3.

INTRODUCTORY EXERCISES IN PHYSICS AND CHEMISTRY

III.—PROPERTIES OF PHOSPHORUS.

Apparatus.—Piece of yellow phosphorus, evaporating dish, forceps, knife.

Directions.—(Caution! Phosphorus should be kept under water and cut under water. It should not be allowed to come in contact with the bare skin.)

Fill the evaporating dish with water and with the forceps place the piece of phosphorus in it. With the knife cut off a piece and examine the cut surface. Does it cut easily? Describe its consistency. What color is the new cut surface? Leave this exposed to the light for a time, keeping it under water, and note any change in color. Is phosphorus soluble in water?

Pick up a piece with the forceps, wipe dry with filter or blotting paper, and hold in the air a moment. Describe what takes place. Why is phosphorus kept under water? Does phosphorus give off any odor?

Rub phosphorus gently on a piece of paper and examine the paper afterwards in the dark. What evidence have you that phosphorus burns at a low temperature?

(Bone is the part of the body richest in phosphorus.)

Make a list of the properties of phosphorus so far as you have observed them.

IV.—PROPERTIES OF SULPHUR.

Apparatus.—Half a teaspoonful of flowers of sulphur or a piece of stick sulphur, a silver spoon, a hard-boiled egg, a raw egg, an evaporating dish, alcohol lamp or Bunsen burner.

Directions.—Examine a little of the sulphur. Has it any odor? taste? color? Shake some up in water. Does it dissolve?

Place a little in the dry evaporating dish and heat gently. Does it melt? Describe its condition. Continue to heat and describe the various changes through which it passes.

Touch a match to a little dry sulphur. Does it burn? Describe the result. Smell of the fumes (Caution!). Where have you noticed this odor before? (This odor is due to a gas called an *oxide of sulphur* and this gas is formed whenever sulphur is burned.)

Place a little of the sulphur in the bowl of the silver spoon. After a moment brush it off. Is the silver still bright? (When silver is brought in contact with sulphur the latter unites with it and forms a compound called *sulphide of silver*, which is black.)

Mince the hard-boiled egg with the handle of the silver spoon. What happens? Compare with above result. (The two stains are identical and the latter indicates the presence of sulphur in eggs.)

Place the raw egg in a clean evaporating dish and leave in a warm place for several days.¹ When the egg decays note the odor. (This odor is due to another compound of

¹ It is well to place the dish in a closed vessel containing a little water, as otherwise the egg may dry up without decaying.

sulphur called *hydrogen sulphide*. When animal flesh decays it gives off this odor, showing that flesh contains sulphur.)

Mention seven properties of sulphur which you have observed in the above experiments.

V.—PROPERTIES OF CARBON.

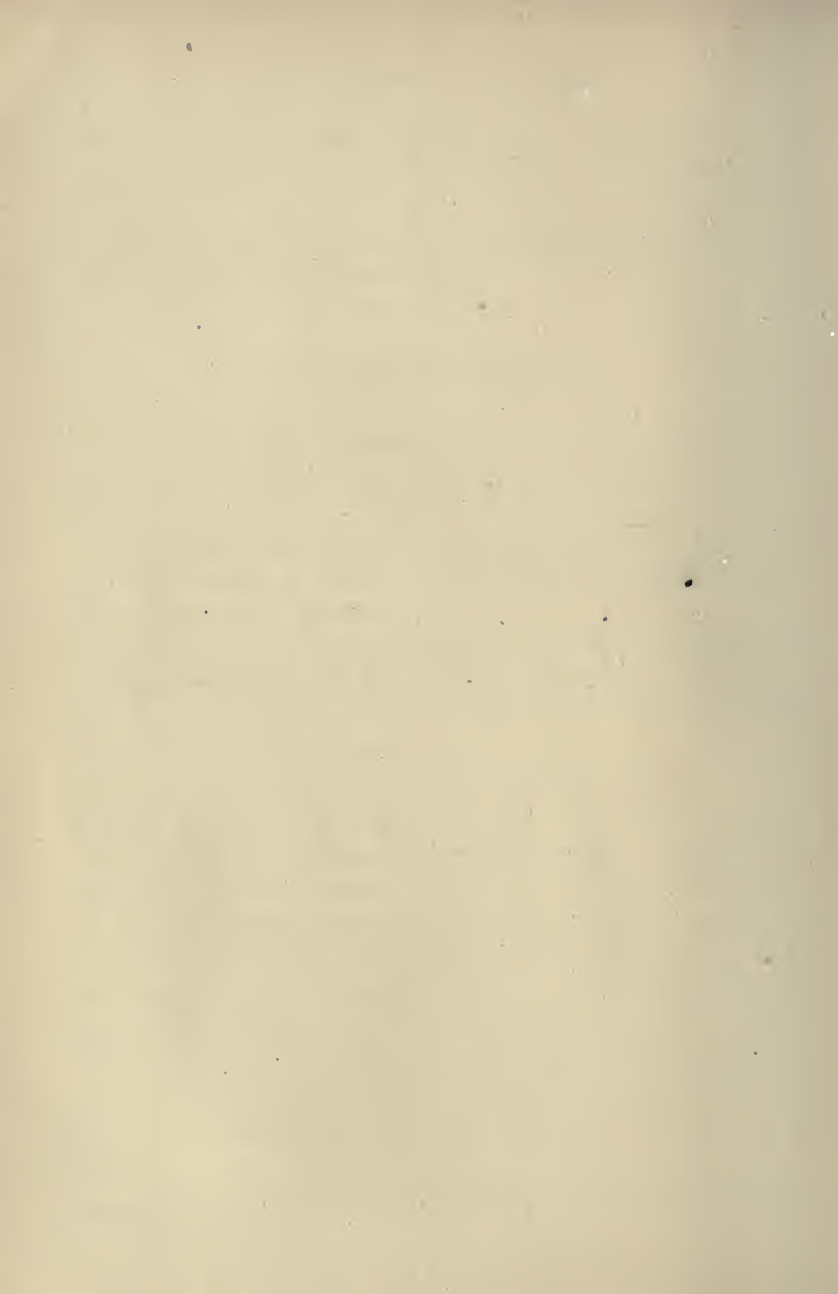
Apparatus.—Stick of wood-charcoal, bottle with a small mouth, limewater, ¹glass tube six or eight inches long, beaker, test tubes, splinter of wood, piece of meat, piece of marble, hydrochloric acid.

Directions.—Examine the charcoal stick. (Charcoal is one of the forms of carbon.) What is its color? odor? taste? Does it dissolve in water?

Light the stick, after trimming it to such a size as to enable it to be thrust through the neck of the bottle. Does it give off any odor in burning? Is it like or unlike sulphur in this respect?

Thrust the lighted stick of charcoal into the bottle and keep it there until the flame goes out. Now remove it and cover the mouth of the bottle with the finger. Can you see anything in the bottle? Take a drop of clear limewater in the end of the glass tube and hold it in the air a few minutes. Is there any change in the limewater? Now introduce it into the bottle without touching the sides of the bottle. What happens to the color of the limewater? What sort of substance must be present in the bottle? (When carbon burns it forms a gaseous compound with the oxygen of the air called an *oxide of carbon* or *carbonic acid*

¹ Limewater may be made by slaking a little quicklime in water and decanting the clear liquid.



gas. This gas is the only one that will cause the change in limewater noted above.)

Rinse out the bottle with water. Light the wood splinter and thrust into the bottle. Proceed as with the charcoal. Test the contents of the bottle with a drop of limewater. What evidence have you that wood contains carbon?

Burn the piece of meat by heating it in the test tube. Suspend a drop of limewater in the tube by means of the glass tube. What evidence have you that animal flesh contains carbon?

Place the piece of marble in a clean test tube. Pour on it a little hydrochloric acid which has been diluted previously with twice its volume of water. What evidence of action have you? Hold suspended a drop of limewater in the mouth of the tube. Hydrochloric acid and water contain no carbon; what must you conclude as to the presence of carbon in the marble?

(Carbon is to be found in all animal and vegetable compounds and in some minerals.)

Pour some of the limewater into the beaker. By means of the glass tube blow some of your breath through the liquid in the beaker. In what form is the carbon in your breath? (Expired air contains about 4 parts of this gas in every 100 parts of the expired air. Ordinary air contains about .04 part of this gas in 100 parts, or about 4 parts in every 10,000 parts of air.)

(Besides charcoal, the other forms of carbon are diamond and graphite. All the forms of carbon are odorless, tasteless, and insoluble in water; and if strongly heated in the presence of oxygen, each form of carbon will combine with it and form carbonic acid gas.)

VI.—PROPERTIES OF IRON.

Apparatus.—Several feet of fine wrought-iron wire, a magnet, an evaporating dish.

Directions.—Bring the magnet in contact with the iron. Raise the magnet. Note that the iron is attracted to it. See if other things are similarly attracted to it.

Place a coil of the wire in a warm, dry place. Place a like coil in the evaporating dish and cover with water. Leave both coils for several days, and then examine them. Note that one of them is covered with a reddish deposit (rust). What conditions are favorable to this formation? (Rust is a compound that iron forms with the oxygen of air and water. It is this power of iron to unite with oxygen that makes it valuable as a part of the blood in the animal body; see Exercise XLIII, *D*, on page 71.)

VII.—OXYGEN AND OXIDATION.

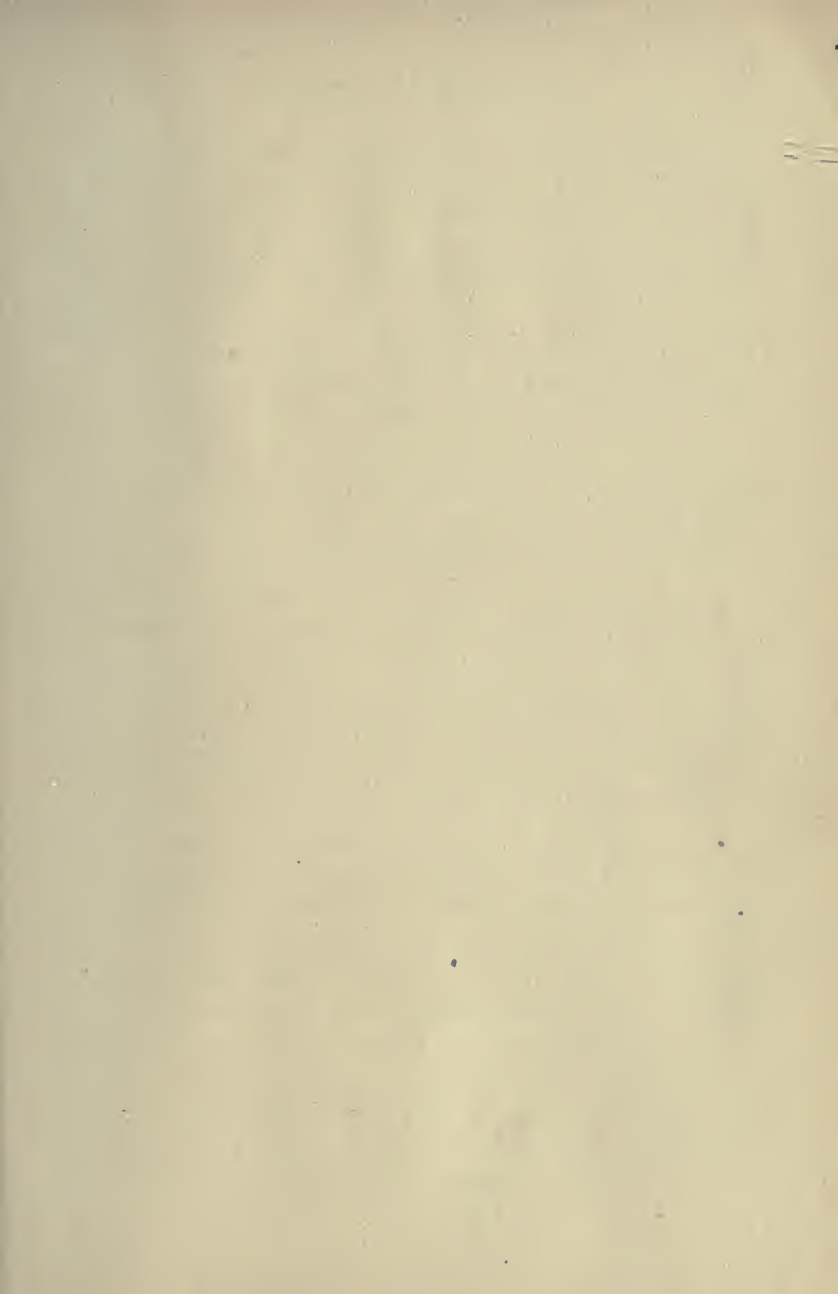
Apparatus.—Red oxide of mercury (mercuric oxide), test tube, stick of charcoal, limewater and glass tube, alcohol lamp or Bunsen burner.

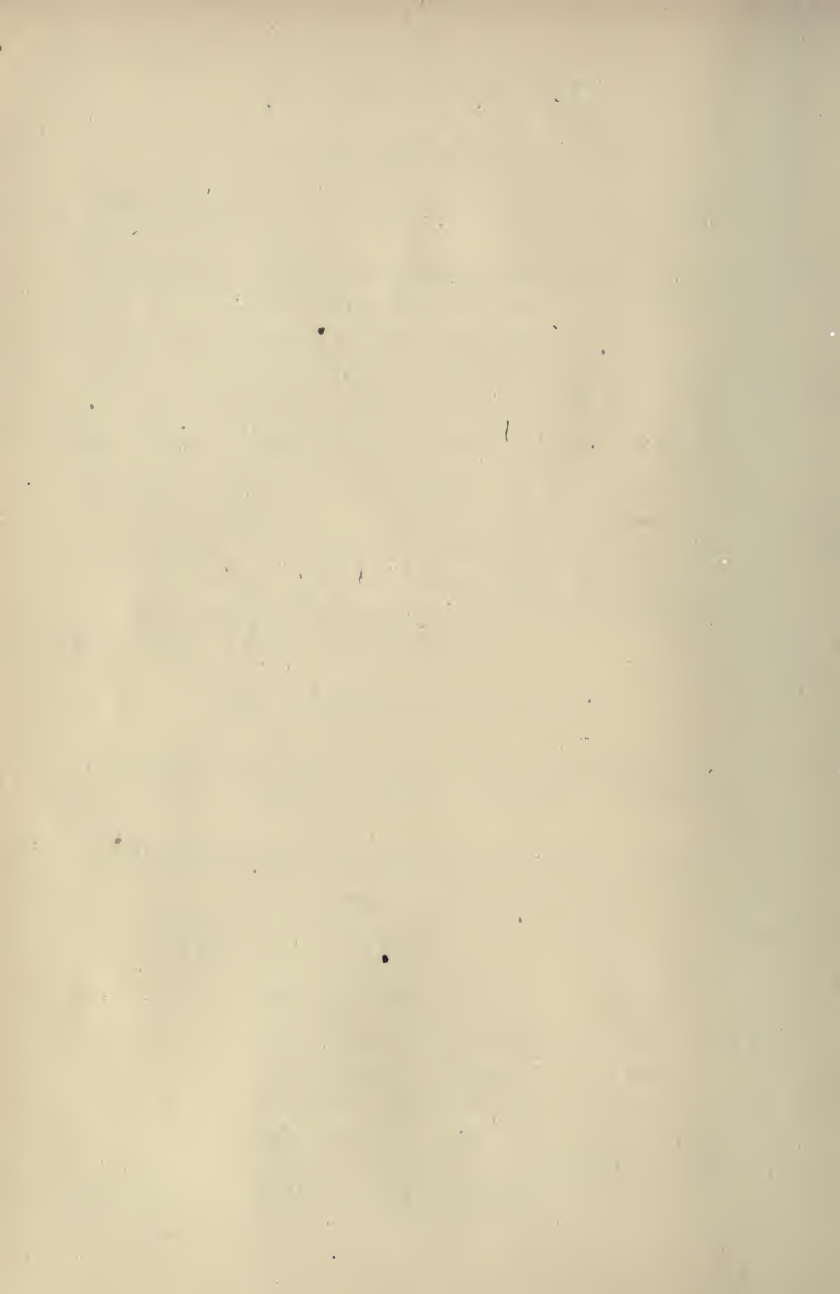
Directions.—Place in a test tube as much red oxide as you can get on your finger nail. Heat the test tube in the flame (see Fig. 4). Heat the end of the charcoal stick until it glows, and introduce it into the mouth of the test tube. After heating the oxide hot you will notice a change in the glow of the charcoal. Describe it. Can you see anything in the tube? If it be a colorless gas that is acting on the charcoal can that



FIG. 4.

gas be air? Reasons for your statement?





Remove the tube from the flame. When the stick ceases to glow remove it and substitute for it a drop of limewater on the end of the glass tube. What happens? What does this indicate? As the tube cools what do you see on the sides of the tube? Do you know the name of this substance?

EXPLANATION. Oxide of mercury is a compound of mercury (quicksilver) and oxygen. Heat decomposes this into oxygen and mercury. In what form were these two substances given off in the above experiment? We have already learned that when charcoal burns it forms a gas called carbonic acid gas. How was this formed in the above exercise? We can express the above actions in the form of equations as follows:

(1) Oxide of mercury + heat = oxygen and mercury.

(2) Oxygen + carbon + heat = oxide of carbon + heat.

In chemical language the process illustrated in (1) is *analysis*, or the separation of a compound into its parts. The process illustrated in (2) is *synthesis*, or the union of parts to make a compound. All chemical actions may be grouped under one or the other of these processes.

The special kind of compound that results from the union of oxygen with a substance is called a compound of oxidation, and the actual formation is called *oxidation*. When oxidation takes place rapidly, light and heat are produced at the same time and the process is called rapid oxidation or *combustion*. Give examples from your experience of both kinds of oxidation—the slow and the rapid. Why does the exclusion of air from a fire cause the fire to go out? What is the precise action of water or sand when thrown on a flame, in the light of the above explanation?

VIII.—PROPERTIES OF OXYGEN.

Apparatus.—Chlorate of potash (potassium chlorate), manganese dioxide, piece of phosphorus, stick of charcoal, sulphur, fine iron wire, Florence flask, one-holed rubber stopper, rubber and glass connecting tubing, wash bottle fitted with two-holed stopper, ring stand, sand bath, pneumatic trough, five large-mouthed glass bottles with glass plates to cover, caustic soda, Bunsen burner or alcohol lamp, deflagrating spoon.

Directions.—Set up the apparatus as in Fig. 5. Place

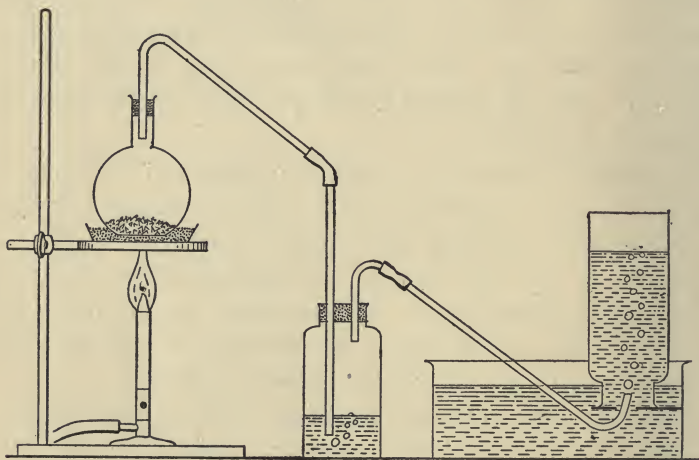


FIG. 5.

in the flask to a depth of half an inch a mixture of one part of manganese dioxide to four parts of chlorate of potash. Fill the wash bottle about half full of water and dissolve a stick of caustic soda in it.¹ When everything is connected as in the diagram heat the flask gently on the sand bath. The first of the gas produced will mix with the air in the

¹ This will absorb the impurities in the oxygen.

apparatus, and should be allowed to escape. When the bubbles of gas flow freely through the delivery tube, fill one of the bottles with water and invert over the delivery tube to receive the gas (oxygen) as in the above diagram (see Exercise II on page 10). When the bottle is full cover with the glass plate and set aside mouth upward. Fill the other four bottles in the same way. Then proceed as follows:

A. Examine the gas in the first bottle. Has it any color? odor? Suck a little into the mouth with a glass tube. Has it any taste?

B. Tie a piece of charcoal to the handle of the deflagrating spoon, heat the end of the charcoal until it glows, and introduce it into the second bottle. Describe the result. Keep lowering the charcoal as it tends to stop burning, until it reaches the bottom of the bottle. Compare this result with that of Ex. VII. What name do you give to this process? How could you test the contents of the bottle to prove your statement? Do so and record result (see Ex. VII).

C. Place in the bowl of the deflagrating spoon a piece of phosphorus the size of a pea (Caution! Handle with forceps and cut under water). Light the phosphorus and introduce quickly into the third bottle. Describe the result. Does it burn more or less brilliantly than in air? Note the white cloud in the bottle. (This is an oxide of phosphorus and is formed by the uniting of the phosphorus and the oxygen.) Compare this result with that in B.

D. After cleaning the deflagrating spoon place some powdered sulphur in it. Light the sulphur. Note how it burns in air and the color of the flame. Now introduce it into the fourth bottle. Describe the re-



FIG. 6.

sult. After the burning is over smell (Caution!) the gas in the bottle. Compare with the odor of burning sulphur in Ex. IV. What is the name of this gas? Is the action noted above combustion? Give your reasons (see Ex. VII.)

E. Heat the end of the fine iron wire red hot and introduce it into the fifth bottle. Describe the result. After the action is over examine the red spots on the sides of the bottle and compare them with the rust obtained in Ex. VI. What is the difference between the two actions?

Name the properties of oxygen that you have observed.

IX.—COMPOSITION OF AIR AND PROPERTIES OF NITROGEN.

Apparatus.—Pneumatic trough, bell jar closed at the top, evaporating dish, test tube, phosphorus.

Directions.—Fill the pneumatic trough so as just to cover the support. Place the evaporating dish on the support. Place in it a piece of phosphorus the size of a pea; light the phosphorus, and cover quickly with the bell jar.

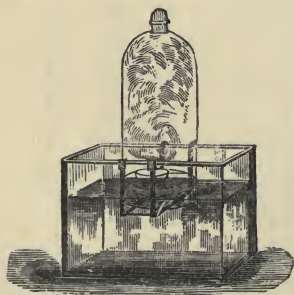


FIG. 7.

A. Note the white fumes that appear. What are these? (See Ex. VIII, *C.*) What is one of the components of air? When the jar is first put on note that some bubbles are forced out because the heat causes the air to expand a little. The phosphorus stops burning when all the oxygen in the bell jar is used up. Let

the apparatus stand until the white oxide of phosphorus has been absorbed by the water and the gas in the jar is clear.

(Phosphorus was used instead of sulphur or charcoal in this exercise because its oxide is a solid which settles and dissolves in the water.) Has the water risen in the jar? What part, by volume, of the jar does it occupy? Since the phosphorus has used up all the oxygen in burning, about what part of air must be oxygen?

B. Fill the test tube with gas from the bell jar in the manner described in Ex. II, *B.* Examine this gas. What is its color? odor? taste? Place a lighted match in it. What happens? Explain. (This gas is called *nitrogen*.¹) Of what advantage is the presence of nitrogen in the air? Why is a good draught necessary to make a fire burn freely? If the body needs to take in oxygen constantly why can we not live in a sealed room?

X.—COMPOSITION OF WATER (OPTIONAL).

Apparatus.—Electrolysis apparatus,² sulphuric acid, four dry cells, splinters of wood, test tubes, pneumatic trough or other dish of water, glass and rubber connecting tubing.

Directions.—Open the two stopcocks and fill the apparatus with water containing 5% of sulphuric acid. When the tubes are full and all air driven out, close the cocks; arrange the four dry cells in series (positive pole of one connected with negative pole of the next, and so on); and connect the positive and negative poles of the series with the

¹Other gases (carbonic acid gas, argon, water vapor) are present in very small proportions.

²For the electrolysis apparatus shown on p. 22 may be substituted simpler forms with nearly as good results. Simple forms are shown in Clark and Dennis's "Elementary Chemistry," page 33; and in Remsen's "Chemistry, Briefer Course."

posts as indicated in Fig. 8. Note what happens. Where do the bubbles form? In which tube do they form most rapidly? What is the ratio by volume of the gases in the two tubes?

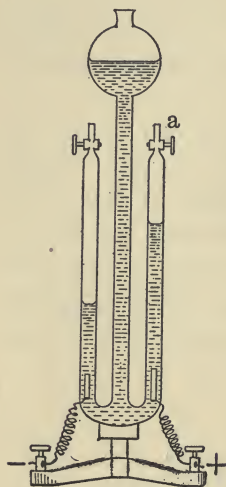


FIG. 8.

When the tube containing the most gas is full, disconnect the cells. Collect in a test tube the gas from the tube containing the lesser amount as follows:

With rubber connecting tubing connect an ordinary delivery tube, filled with water, to the top of the gas tube. Insert the end of the delivery tube into the mouth of the test tube, after filling the test tube with water and inverting as in Ex. II. Open the cock and collect the gas as in Fig. 9. Cover the mouth of the tube with the thumb and hold mouth upward. Now re-

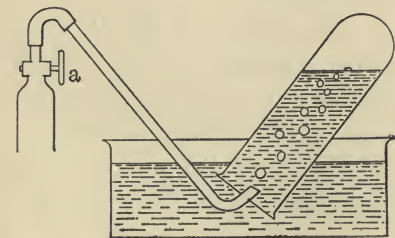
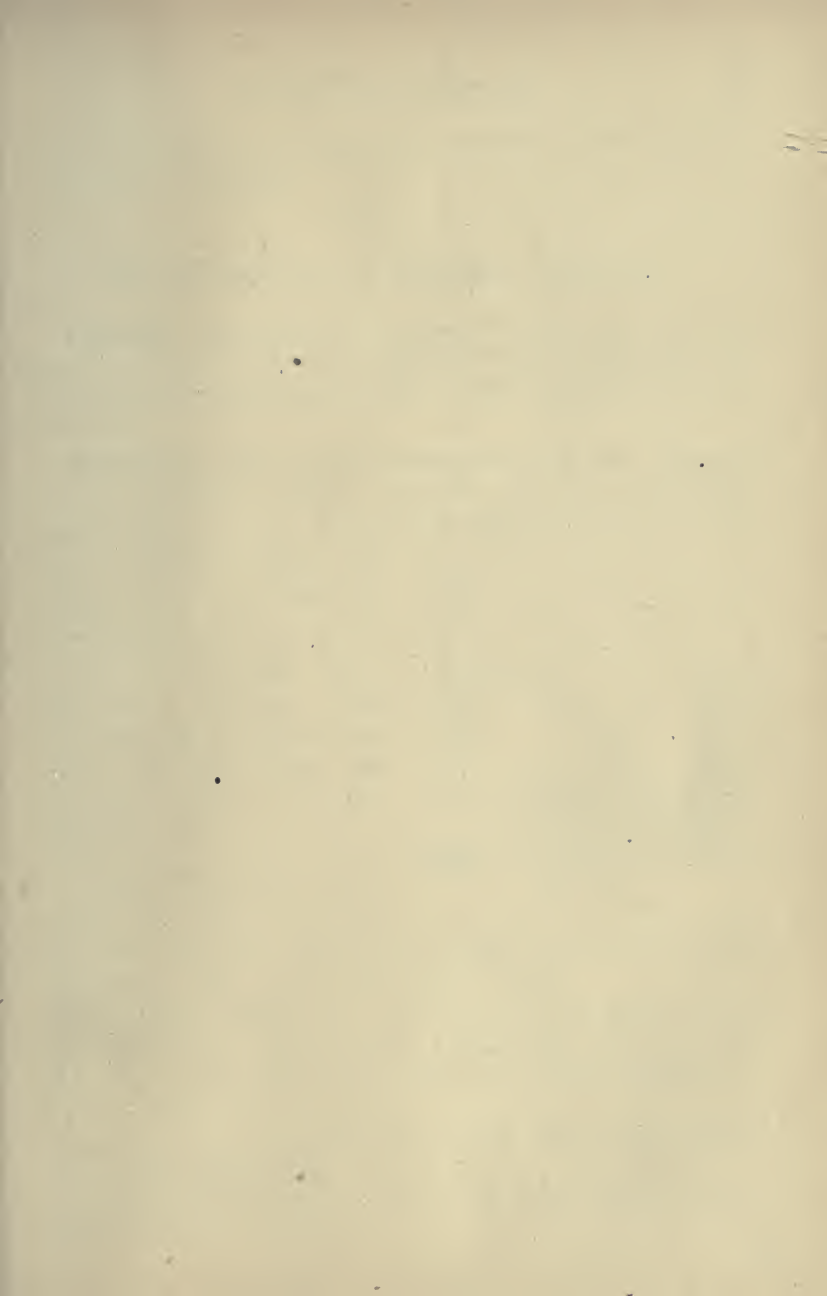


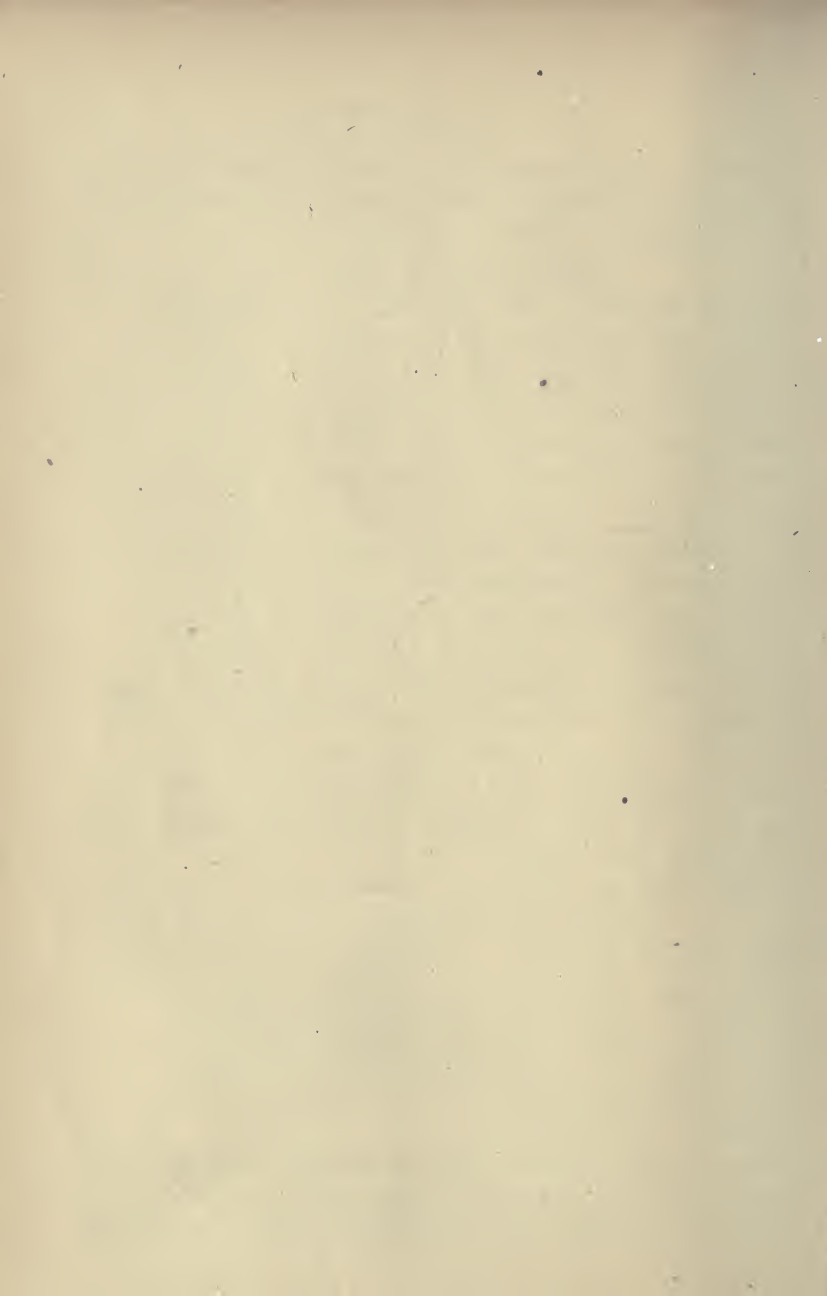
FIG. 9.

remove the thumb and quickly insert a lighted splinter into this collected gas. What happens? What gas have you studied that produces a similar action? This is the same gas.

In a second test tube collect the gas in the other tube. Hold it mouth downward, and introduce a lighted splinter into it. Describe

what happens. How is this gas different from oxygen? from nitrogen? (The name of this new gas is *hydrogen*. The electric current has broken the compound—water—





into its two parts, hydrogen and oxygen.) Is this exercise synthesis or analysis?

XI.—PROPERTIES OF HYDROGEN (OPTIONAL).

Apparatus.—Granulated zinc or pieces of sheet zinc, dilute sulphuric acid,¹ bottle with two-holed stopper, thistle tube, glass and rubber connecting tubing, pneumatic trough, large-mouthed bottle, test tubes.

Directions.—Set up the apparatus as in the diagram. Place a handful of zinc in the bottle and pour on enough dilute sulphuric acid through the thistle tube to cover the zinc. (Caution! Keep all flames away from the apparatus until the gas is collected.) Let this gas escape until it is bubbling freely from the delivery tube; then collect the large bottle full through water as in Ex. VIII.²

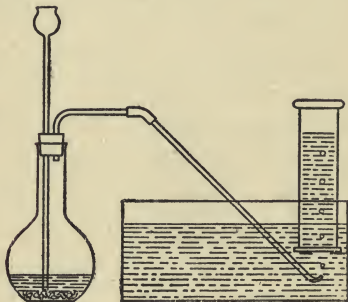


FIG. 10.

A. By the method of Ex. II, B, take some of the gas in a test tube and examine it, holding the test tube mouth downward. Has it any color? odor? taste?

B. Collect a second test tube full and hold mouth downward as before. Tie a match to a wire, light the match, and thrust it up into the tube. Does the match continue to

¹ To dilute sulphuric acid, pour slowly one part of acid into five or six parts of water. Stir while pouring.

² The gas will be freer of impurities if passed through a wash bottle containing permanganate solution.

burn? Reason? Where does the hydrogen burn? Why? After the hydrogen has burned up, examine the sides of the tube. What do you find on them? Why should you expect this? What is oxide of hydrogen?

C. Hold a fresh test tube full of hydrogen mouth upward for a few moments. At the end of that time test with a match. Is the hydrogen still there? Explain. (Hydrogen is the lightest substance known.)

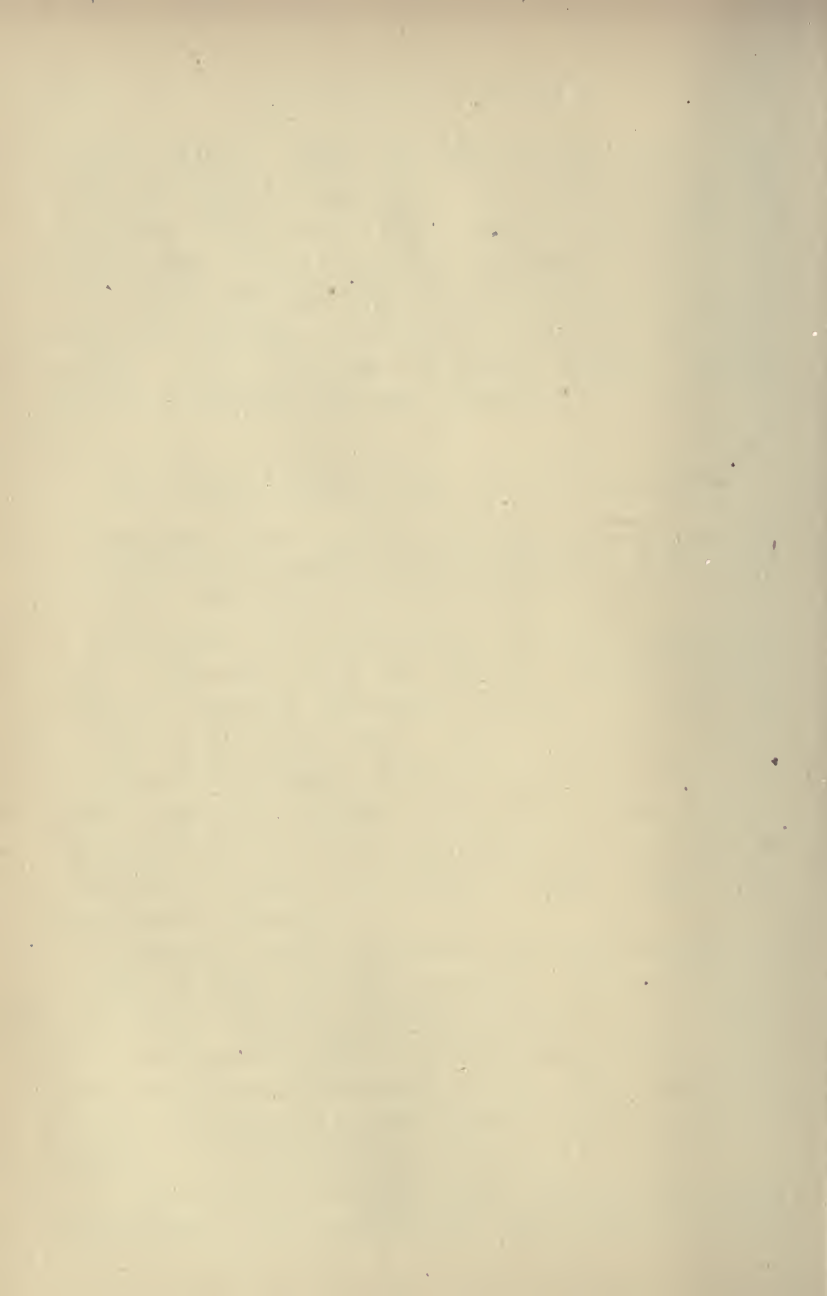
XII.—ACIDS, BASES, SALTS, AND NEUTRALIZATION.

Apparatus.—Dilute hydrochloric and nitric acids (one part acid to ten parts water), caustic soda, red and blue litmus paper, evaporating dish, glass stirring rod, Bunsen burner.

Directions.—*A.* Examine some of the dilute hydrochloric acid. What sort of an odor has it? Describe its taste. Rub some between the fingers; describe its “feel.” Dip a piece of red litmus into it. What is the effect? Dip in a piece of blue litmus. Describe the result. (The taste, “feel,” and effect on litmus noted are three ways in which you can detect any acid.) Test some common substances and record results; *e. g.*, cream of tartar, vinegar, soda, fruit juices, ammonia.

B. Dissolve a stick of caustic soda, an inch long, in a tumbler of water. Examine this liquid. What is its taste? odor? “feel”? Test it with the two kinds of litmus paper and record results. (This kind of substance is called a *base*. Bases always react in this way to taste, “feel,” and litmus. Certain strong bases are called *alkalis*.) Test the substances named in *A*. Which of these are bases?

C. Pour some of the caustic soda solution into the evaporating dish. Add, gradually, the dilute hydrochloric acid,



stirring with the rod and testing with the litmus until the solution turns neither red litmus blue, nor blue litmus red. If too much acid is added correct it with more basic solution. The acid and the base are now said to be *neutralized*, and the process is called *neutralization*. Evaporate this mixture to dryness over the flame. What sort of substance is left in the dish? Taste it. Is it familiar? Does it affect litmus in the solid state or when dissolved in water?

D. Repeat the above neutralization, using nitric acid instead of hydrochloric. Does the product affect litmus?

(The products of *C* and *D* are called *neutral salts*. To this class of substances belong most of the minerals of the earth.) This exercise may be continued with other acids and bases at the desire of the experimenter.

STUDY OF NUTRIENTS

Phosphorus, sulphur, carbon, iron, oxygen, nitrogen, and hydrogen are a few of the chemical elements to be found in plant and animal bodies. These elements occur, however, not as elements, but in combinations, or compounds. There are many of these combinations, but they may be grouped together under a few class names. These classes of compounds show certain definite qualities by means of which their presence may be detected. The classes are called *proximate principles*, or *nutrients*. The most important are:

Proteids, or nitrogenous compounds.

Carbohydrates, or starches and sugars.

Fats and oils.

Mineral salts.

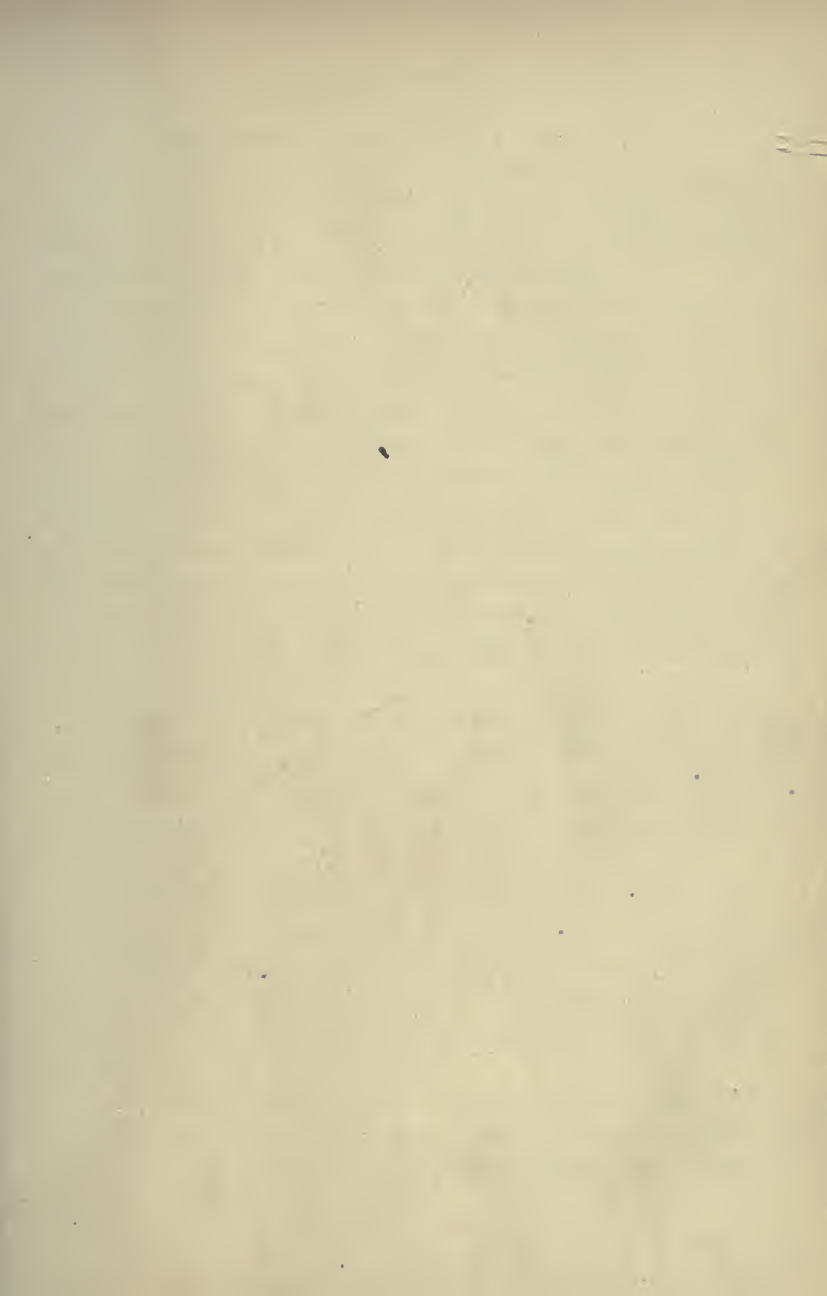
Water.

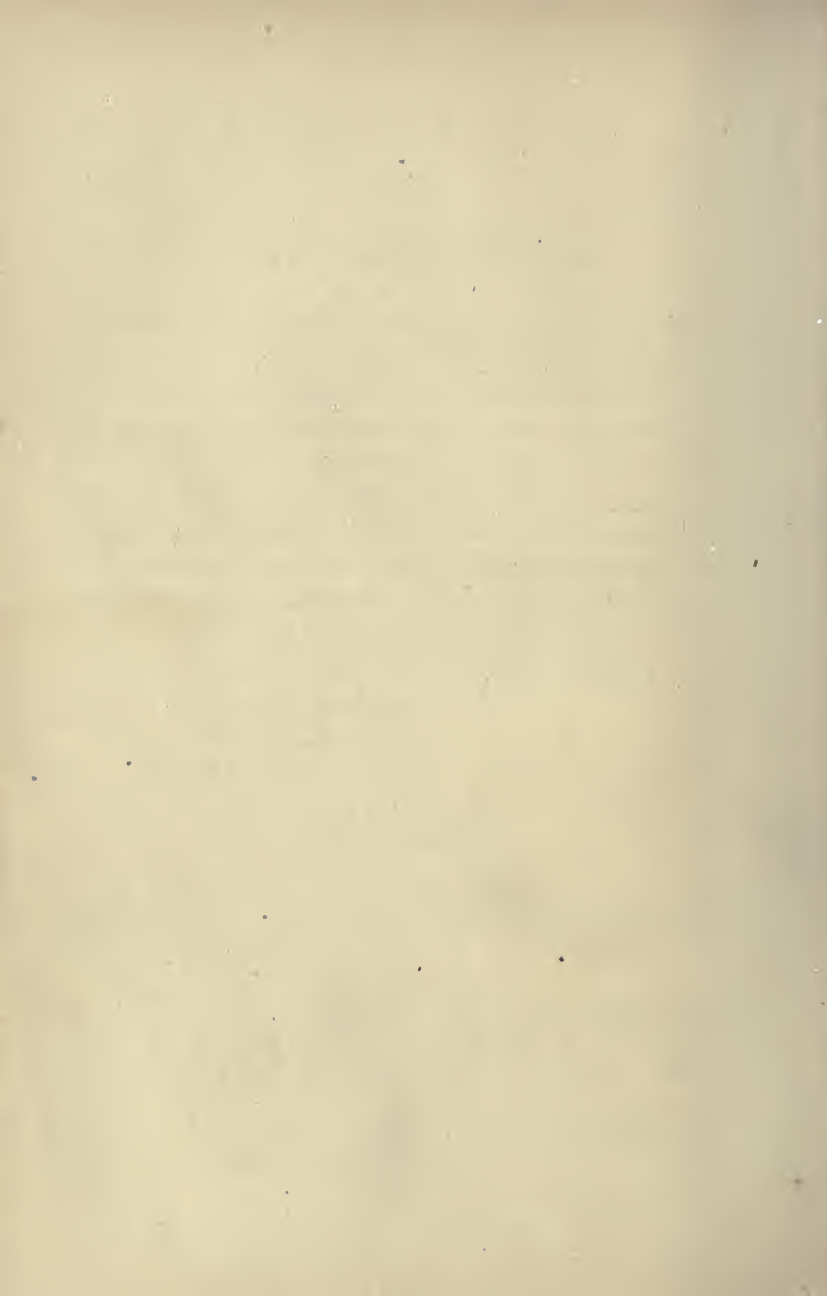
XIII.—PROTEIDS.

Apparatus.—Raw white of egg, olive oil, salt, nitric acid, ammonia, Millon's reagent,¹ caustic soda, sulphate of copper (blue vitriol), test tubes.

Directions.—A. Put a little white of egg (a good example of proteid) in a test tube, cover with two inches of water, and shake. Does the white of egg dissolve? Heat the

¹ To make Millon's reagent, mix one part of mercury by weight with two parts of nitric acid (concentrated commercial); when the mercury is all dissolved, dilute with twice the volume of water.





water and egg mixture slowly. What form does the white of egg take now? Is this form soluble in water?

Put a second portion of the egg in a test tube. Add dilute nitric acid to it. What happens to the white of egg? Compare the action with that in boiling water. *

(This action of acid and heat on a proteid like egg albumin is called *coagulation*.) Why does a piece of meat (which is composed mainly of a proteid like white of egg) become more solid under heat?

B. Xanthoproteic Test. Place a little coagulated white of egg in a test tube and cover with dilute nitric acid. Heat to boiling and then add enough ammonia to neutralize the acid and give an alkaline test. The white of egg (proteid) takes what color? Treat in the same way some olive oil, some common salt, and any other substance that does not contain proteid. Do any of these take the same color as the white of egg?

C. Millon's Test. Add enough Millon's reagent to a little coagulated white of egg to cover, and boil. What color does the egg and the solution become? Treat the other substances mentioned in *B* in the same way. Do they act like the egg?

D. Biuret Test, or Piotrowski's Reaction. Add caustic soda in concentrated solution to some white of egg, in a test tube. To this add a few drops of a solution of copper sulphate. What color do you get? Boil. What change takes place in the color? Test other substances mentioned in *B* in the same way.

(Of the three chemical tests for proteid given above, the xanthoproteic is best for general use. There are many forms of proteid, but these tests will indicate its presence whatever its form may be.)

XIV.—CARBOHYDRATES—STARCH.

Apparatus.—Solution of iodine,¹ laundry starch, white of egg, olive oil, test tubes.

Directions.—Place a little starch in a test tube and fill the tube a quarter full of water. Shake it. Does the starch dissolve? Boil; what happens to the starch?

Put a little of the starch paste in a test tube with an inch of water. Boil. Now add a drop of the solution of iodine. What color does the paste become?

Test a little white of egg and olive oil (which contain no starch) in the same way. Do you get the same result?

(This test will indicate the presence of starch, whatever may be its form.)

XV.—CARBOHYDRATES—GRAPE SUGAR.

Apparatus.—Fehling's solution,² raisins, starch, oil, test tubes.

Directions.—Mince the raisins, cover with water in a test tube, and let stand until the grape sugar (glucose or dextrose) has dissolved. Put a little of the clear solution in a second test tube and dilute it with double its volume of water. Add a few drops of Fehling's solution and heat to boiling. When no further change in color

¹ To make the iodine solution, dissolve a teaspoonful of potassium iodide crystals in a tumbler of water. Add crystals of iodine and stir until a rich wine color is obtained. This may be bottled and used as needed.

² To make Fehling's solution:

A. Dissolve 35 grams of copper sulphate in 500 c.c. of water. Label this solution (A).

B. Dissolve 160 grams of caustic soda and 173 grams of Rochelle salts in 500 c.c. of water. Label this solution (B).

Keep these two solutions separate until ready for use. Prepare for test by mixing equal quantities.

takes place, note the final color. Test in the same way oil, starch, and any other substance that contains no grape sugar. Compare results.

(This is a universal test for grape sugar.)

XVI.—FATS AND OILS.

Apparatus.—Flaxseed (ground), beef fat, unglazed paper, benzine, filter paper, glass funnel, evaporating dish, chemical thermometer.

Directions.—*A.* Put a little beef fat in the evaporating dish and heat. When it begins to melt stir with the chemical thermometer and note the temperature of the melting point. If the body temperature is 98° F. what does this experiment indicate as to the condition of fats in the body? Name some fats that are liquid at ordinary temperatures.

B. Place a little beef fat on the unglazed paper and warm. Remove and examine the paper. How does it show the presence of fat? Substitute for the beef fat a little ground flaxseed and repeat the above process. Does flaxseed act like beef fat? Do starch and other substances which contain no fat or oil, act in the same way? (The above is a general test for fats and oils in whatever form they may be.)

C. Place a teaspoonful of ground flaxseed in the evaporating dish and cover with benzine. (Ether will act in the same way.) Cover the dish and allow it to stand for a few hours. At the end of that time, filter off the benzine by means of the funnel and filter paper, into a clean evaporating dish. Allow this dish of filtered benzine to stand for a time until all the benzine has evaporated. What is

left in the dish? What did the benzine do to the fat in the flaxseed? Treat sugar or anything else that contains no fat or oil in the same way. Is the result the same? (The above method is another test for fat or oil.)

XVII.—MINERAL SALTS (OPTIONAL).

Apparatus.—Platinum foil or piece of sheet iron, forceps, piece of meat or vegetable matter.

Directions.—Place the meat on the foil and hold the foil in the flame with the forceps until all the black has disappeared from the burning meat. The residue is mineral matter. Would this test be possible if this mineral matter were combustible? What color is the residue? (This is the test for determining the presence and amount of mineral salts.)

XVIII.—WATER (OPTIONAL).

Apparatus.—Pieces of parsnip, potato, apple, lettuce leaves, flour, meal, meat, test tube, balance sensitive to one gram.

Directions.—A. Heat one of the above substances in a dry test tube. As the tube cools after having been taken from the flame, examine the sides and note what you see on them. In what form was the water before the tube was cooled?

B. Weigh a portion of each of the above substances, record the weights, and place the substances in a warm, dry place for a few days. Then weigh again and record as before. Continue this until there is no further decrease in

weight. The loss of weight represents approximately the water contained in the substances before it evaporated. From your results answer the following questions: About what per cent of water did each substance contain? Why are flour and grains in general a good food for travelers to carry? Why are fruits and salads good hot-weather foods?

STUDY OF FOODS

XIX.—NECESSITY OF FOOD.

Apparatus.—Wide-mouthed bottles, corks to fit, pea or corn seedlings, nutrient solution,¹ test tubes, paraffin wax, distilled water.

Directions. A. Take one of the pea or corn seedlings and cut off the cotyledons close to the stem. Pass this through a hole in one of the corks, and insert in a bottle as shown in Fig. 11. Fill the bottle about three quarters full of the nutrient solution. Prepare a second seedling in the same way (select one of as near the same size as possible), but substitute distilled water for the nutrient solution. Note the growth of each seedling for several days. Do they grow equally fast? What sort of food is in the nutrient solution? From the composition of the



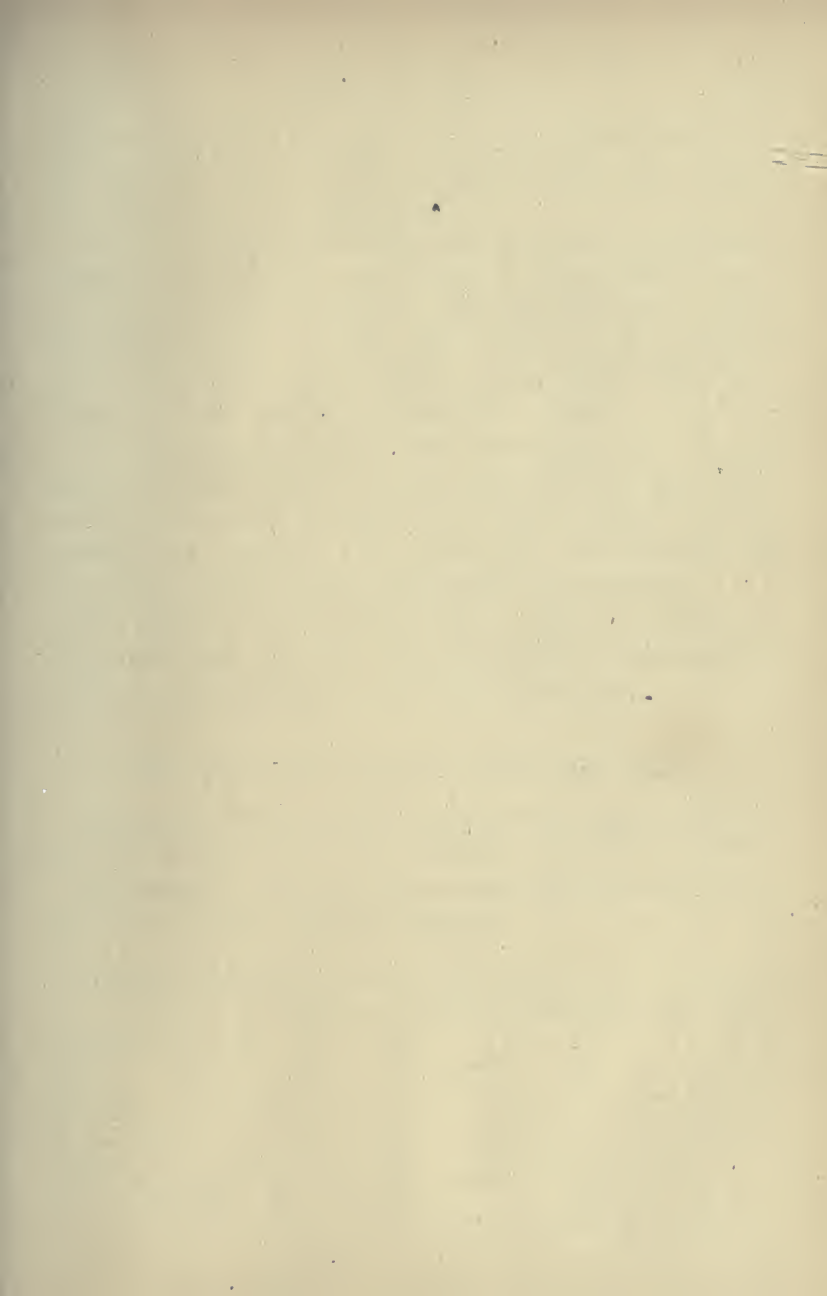
FIG. 11.

water and the mineral salts, is it possible for the plant to get its carbon from the nutrient solution? (Air contains a small proportion of carbonic acid gas [see Ex. V] and plants

¹Nutrient Solution after Sachs ('82).

Distilled water (H_2O).....	1000.00	c.c.
Potassium nitrate (KNO_3).....	1.00	gram
Sodium chloride ($NaCl$).....	0.50	"
Calcium sulphate ($CaSO_4$).....	0.50	"
Magnesium sulphate ($MgSO_4$).....	0.50	"
Calcium phosphate ($Ca_3 [PO_4]_2$).....	0.50	"
Ferric chloride ($FeCl_3$).....	0.005	"

(Do not put the ferric chloride into the solution in the first place, but add a drop of it to each bottle when the seedlings are put in.)



secure their carbon through their leaves, which absorb the gas from the air.) From your observations can a plant live indefinitely on water alone?

B. Seal the growing seedling of *A* to the cork with melted paraffin wax, cover the upper part of the seedling with a test tube, and seal the edges of the tube to the cork. Does the plant continue to live? (Plants can not live without air.) What collects on the inside of the tube? What evidence have you that a plant gives off water?

C. Prepare a third and a fourth seedling as in *A*. In one bottle put some nutrient solution made without the potassium nitrate, and in the other the normal solution. Which seedling lives longest? (To support life, one food required by plants is some nitrogen salt like potassium nitrate.)

D. Prepare a fifth seedling as in *A*, using a nutrient solution with only the ferric chloride omitted. After several weeks, notice the effect on the color of the plant.

XX.—NUTRIENTS PRESENT IN COMMON FOODS.

Apparatus.—Meat, flour, milk, parsnip, lettuce, peanuts, and the necessary materials for the tests described in Exs. XIII to XVIII.

Directions.—Apply the tests to each substance separately and tabulate your results as follows:

Substance Tested.—.....

TEST	RESULT	NUTRIENT PRESENT	RELATIVE AMOUNT
Xanthoproteic			
Biuret			
Millon's			
Iodine sol.			
Fehling's			
Unglazed paper			
Benzine			
Dry test tube & heat			
Platinum foil & heat			

Under "Result" tell exactly what happens.

Under "Nutrient Present" write the name of the nutrient that the test shows to be present. If no reaction follows from the test leave this space blank.

Under "Relative Amount" write *Little* if the reaction is weak, and *Much* if the reaction is strong.

From your results, why are flour, meat, and milk considered especially valuable foods?

XXI.—STUDY OF FOOD CHARTS.¹

Food	COMPOSITION % OF NUTRIENTS						Energy in Calories per Pound	Average Cost per Pound
	Proteid	Starch	Other Carbo- hydrate	Fat	Water	Mineral		
Bread (White)	8.	47.	3.	1.	37.	2.	1280.	\$.04
Flour	11.	66.	4.2	2.	15.	1.7	1645.	.025
Oatmeal	12.6	58.	5.4	5.6	15.	3.	1850.	.05
Rice	6.	79.	0.4	0.7	13.	0.5	1630.	.07
Beans	23.1	55.	2.	2.	12.6	3.1	1615.	.05
Potatoes	2.	18.	3.	0.2	76.	0.7	375.	.0125
Milk	4.	—	5.0	4.0	86.	0.8	325.	.035
Cheese	28.3	—	1.8	35.5	30.2	4.2	2070.	.16
Beef (Round)	20.5	—	—	10.1	68.2	1.2	805.	.14
Beef (Corned Flank)	14.2	—	—	33.	49.8	3.	1655.	.10
Mutton (Leg)	18.3	—	—	19.	61.8	0.9	1140.	.18
Veal (Shoulder)	20.2	—	—	9.8	68.8	1.2	790.	.20
Pork (Shoulder—fresh)	16.	—	—	32.8	50.3	0.9	1680.	.16
Pork (Ham)	16.7	—	—	39.1	41.5	2.7	1960.	.16
Pork (Salt Fat)	0.9	—	—	82.8	12.1	4.2	3510.	.12
Chicken	24.4	—	—	2.0	72.2	1.4	540.	.20
Eggs	14.9	—	—	10.5	73.8	0.8	721.	.18
Butter	1.	—	0.5	85.	10.5	0.3	3615.	.30
Cod fish	15.8	—	—	0.4	82.6	1.2	310.	.08
Mackerel	18.2	—	—	7.1	73.4	1.3	640.	.12
Oysters	6.	—	3.7	1.2	87.1	2.	230.	.25

¹ More extensive tables may be found in a pamphlet printed by the Department of Agriculture, Farmer's Bulletin No. 23, "Foods, Nutritive Value and Cost," by W. O. Atwater.

DIETARY STANDARDS.

CONDITIONS	PROTEID	CARBO- HYDRATES	FAT	CALORIES
Man with light muscular exercise.	0.22 lbs.	0.88 lbs.	0.22 lbs.	2980.
Man with moderate " "	0.28 lbs.	0.99 lbs.	0.28 lbs.	3520.
Man with active muscular work.	0.33 lbs.	01.10 lbs.	0.33 lbs.	4060.

Questions to be answered from study of Food Chart.

Fat and carbohydrates are the energy producers: how does the table show this? What sorts of foods are richest as proteid furnishers (tissue builders)? Of the animal and vegetable foods, which are richest in proteid? fat? carbohydrates?

Calculate the cost, amount of energy in calories, and per cent of nutrients, in the following daily dietaries:

(a) 13 ounces of beef (round), 3 ounces of butter, 6 ounces of potatoes, 22 ounces of bread.

(b) 4 ounces of salt pork, 2 ounces of butter, 16 ounces of beans, 8 ounces of bread.

(c) 10 ounces of beef (corned), 1 ounce of butter, 16 ounces of milk (pint).

Make up a suitable daily dietary for each of the three different classes of men given in the table.

HISTOLOGICAL STUDIES

XXII.—PARTS OF A CELL.

Apparatus.—Scalpel, compound microscope¹ with two-thirds and one-sixth inch objectives and one inch ocular, glass slides and cover glasses, piece of filter paper, methyl green or Delafield's hæmatoxylin.²

Directions.—Sterilize the scalpel by holding it in boiling

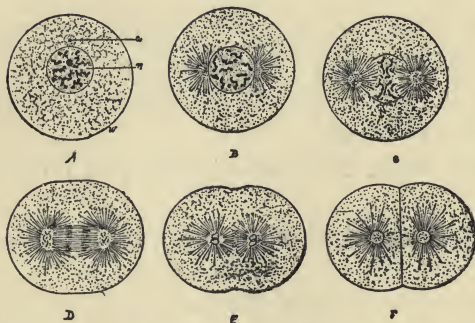


FIG. 12.—A, diagram of a cell; *w*, cell wall with inclosed cytoplasm; *n*, nucleus, consisting of nuclear membrane inclosing granular substance, in which are seen a spherical nucleolus and irregular masses of chromatin; *a*, centrosome; B-F, changes that take place during cell division.

water, then scrape the inside of the cheek lightly with the blade. When the scalpel is removed from the mouth there will appear on it the scrapings in the form of a white sediment. Remove a little of this sediment and mount in a drop

of water on the slide. Cover with the cover slip and examine with the two-thirds objective (low power). In focus-

¹ Bausch & Lomb and the Spencer Lens Co. furnish at request a pamphlet describing all the parts of the microscope and method of handling the instrument.

² For the preparation of these stains consult any manual of microscopy. Lee's "Vade Mecum" is recommended.

ing, the best results are obtained if nearly all the light is excluded by the diaphragm.

Draw what you see. Note that the masses are made up of separate elements (*cells*). Compare with Fig. 12, A. Are the walls circular as in the figure?

Place a drop of the methyl green at one side of the cover slip and by placing the filter paper at the opposite side draw this solution under the slip. Let the slide stand for a moment and examine again with the low power. What part of the cell has changed color? (This part is called the *nucleus* of the cell.)

Now focus on one of these cells with the one-sixth objective (high power). Has the cell a definite outline? Note the clear liquid between the nucleus and the outline. Do you notice any particles floating in this liquid? Draw this cell, magnified to an inch diameter, and label as follows: the outside boundary, or *cell wall*; the clear liquid, or *protoplasm*; the particles floating in this protoplasm, or *granules*; the *nucleus*.

XXIII.—STUDY OF A PLANT CELL.

Apparatus.—Pond scum (*Spirogyra*), normal salt solution,¹ materials described in Ex. XXII.

Directions.—Mount a little of the pond scum in a drop of water and cover with a glass. Examine with the low power. Do you see any separate units in this case? How are they arranged? What is their color? Is this color evenly distributed throughout the cell or located in definite parts of the cell? Can you see any cell wall? protoplasm?

¹ Normal salt solution is made by adding six-tenths of one per cent of common salt (NaCl) to distilled water.

nucleus? Make a drawing of what you see and label in such a way as to answer the above questions.

Now add a little of the normal salt solution, to be run under the cover glass, and examine with the high power. Do you see any nucleus now? any protoplasm? What has happened to the protoplasm? Draw and label such parts of the cell as show. A little methyl green or Delafield's hæmatoxylin added will make the nucleus more distinct.

Make a list of the differences and similarities between the cells examined in Ex. XXII and Ex. XXIII.

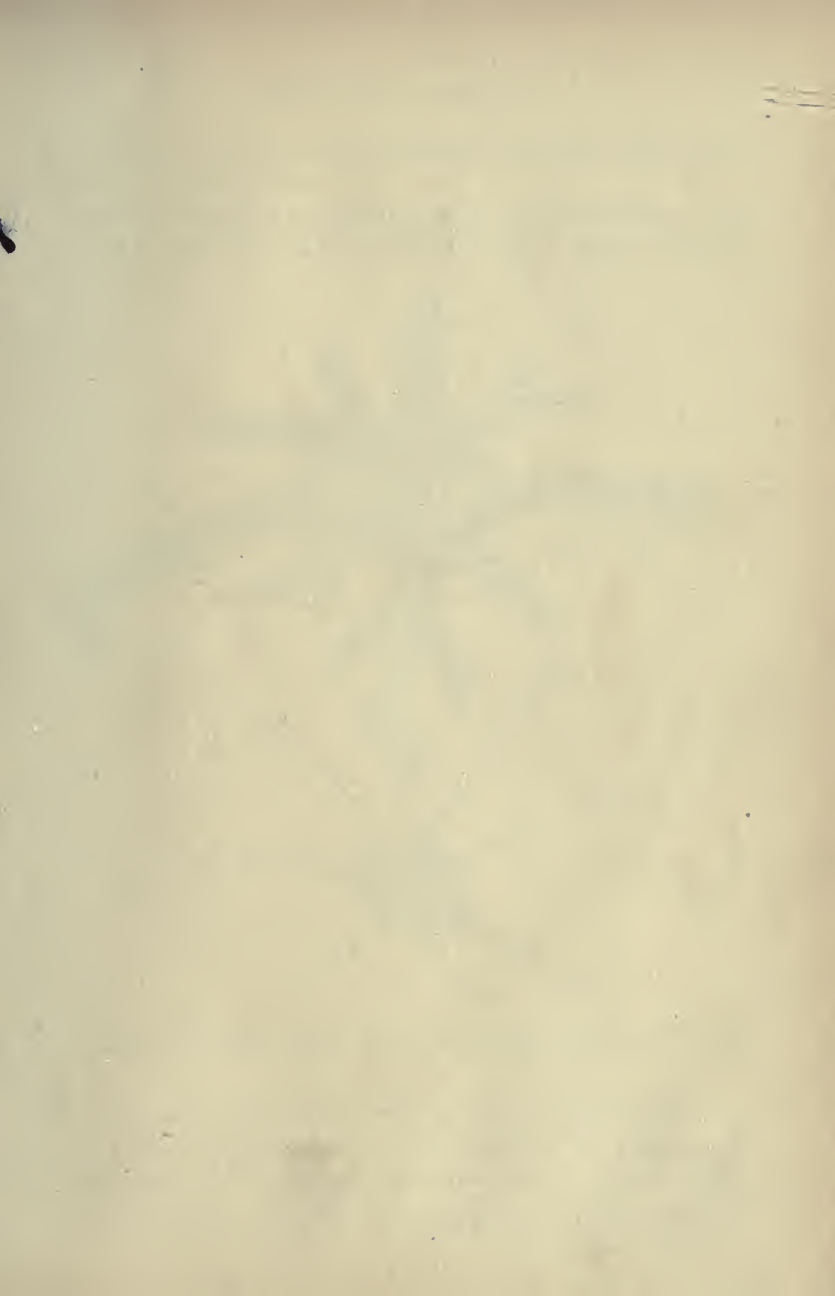
NOTE.—The comparison of cells should be further demonstrated with other materials by the instructor, until the essential and variable components are clearly grasped by the pupil. Some suggested material: *Pleurococcus*, potato, diatoms, root tips, etc.

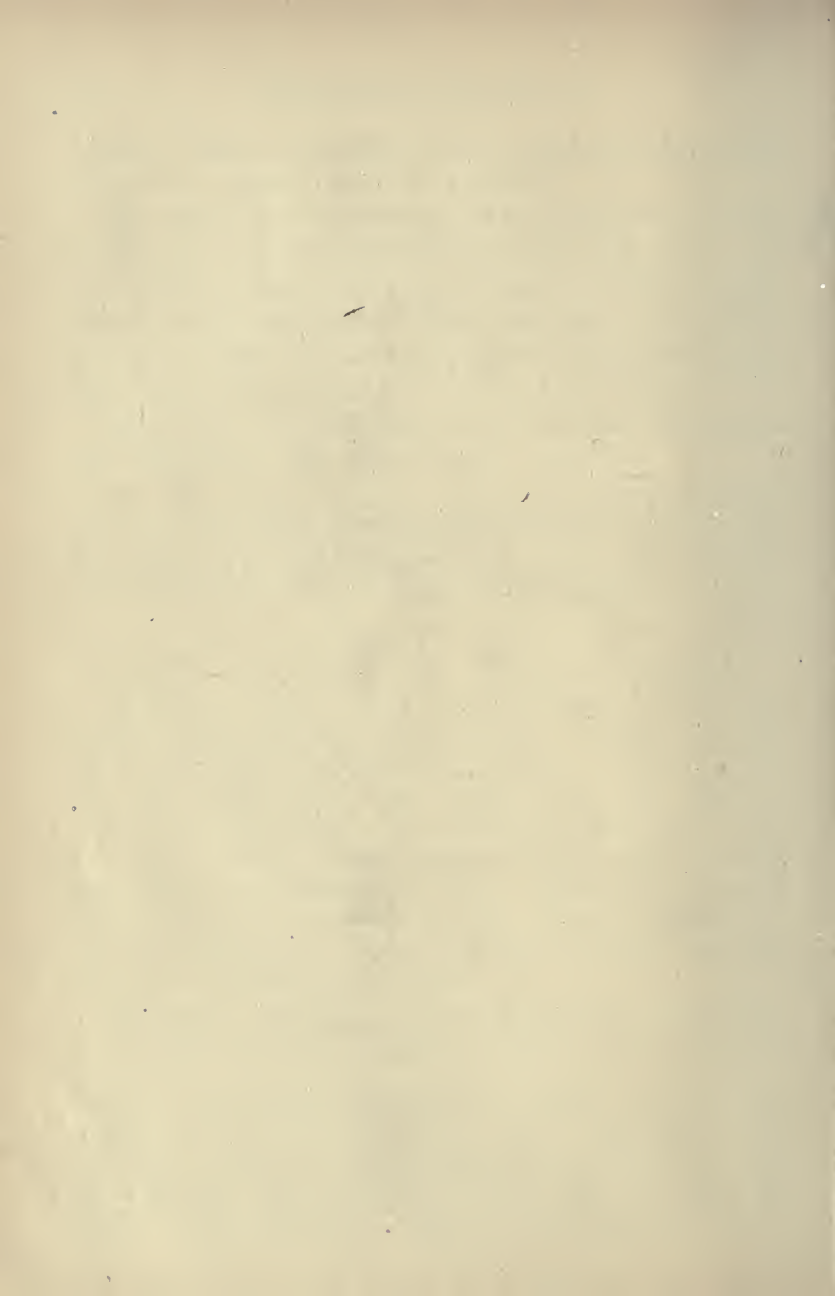
XXIV.—STUDY OF LIVING PROTOPLASM—AMŒBA.

Apparatus.—About a month beforehand collect the leaves and sediment from pools of still, but clear water. Distribute this material—together with a few water plants (*Nitella* or *Chara*)—in several open, shallow dishes. Keep covered with water. When, in course of time, the water in these has become clear and free from scum, take up with a pipette (medicine dropper) some of the sediment from the very surface of the leaves. Examine this for amœbæ with the low power (two-thirds objective). When the dish containing them in quantity is located, mark this for supply.¹ The other apparatus is the same as in Ex. XXII.

Directions.—Mount some of the amœbæ on a glass slide, and cover them with a cover slip. Locate one of the animals

¹ A. W. Weyssse of Boston University gives in "Science," Vol. XX, No. 515, the following method of securing amœba. Collect a considerable number of lily pads. Remove with a spatula the slime which adheres to the lower surface and put it in a shallow glass aquarium containing water six or eight centimeters deep. Place the vessel near a window and in a week or two amœbæ will be abundant on the surface of the sediment at the bottom.





with the low power and then focus on it with the high power for careful observation.

Watch the amœba until it begins to show movement, then draw and note the following parts: round, opaque *nucleus*,

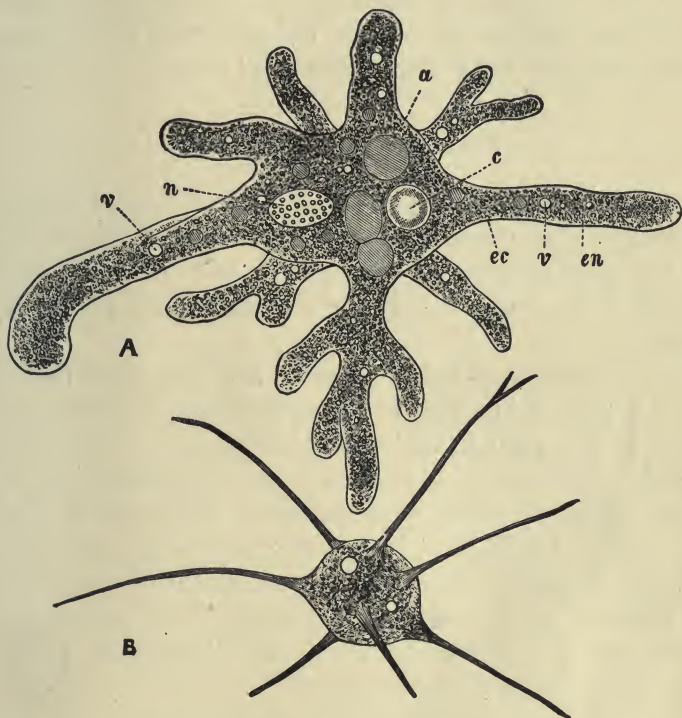


FIG. 13.—A, *Amœba proteus*: a, food vacuole; c, contractile vacuole; ec, ectoplasm; en, endoplasm; n, nucleus; v, water vacuoles. B, *Amœba radiosa*.

the clear outer part (*ectoplasm*) and the granular inner part (*endoplasm*) of the *cytoplasm*. (Cytoplasm is the name given to that part of the protoplasm which is not nuclear, since the nucleus is also composed of protoplasm.) Note, further,

the round spots in the cytoplasm (*vacuoles*: food vacuoles, water vacuoles, or contractile vacuoles, according to contents); the constantly forming projections of the cytoplasm (*pseudopodia*); and the absence of any cell wall.

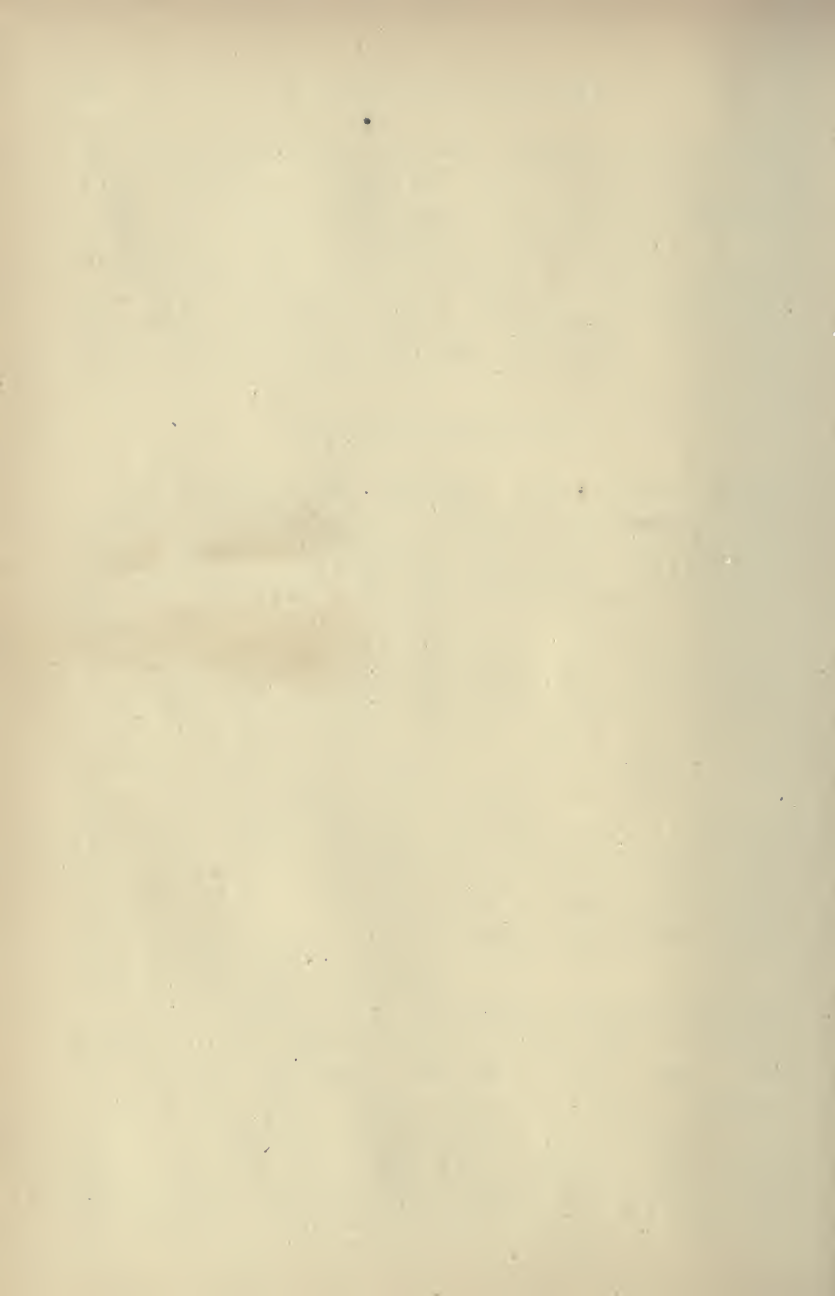
(Amœba is a one-celled animal made up of free protoplasm and hence well suited to show the properties of this substance, which is the physical basis of all life.)

Properties of Protoplasm.

A. What color is the cytoplasm? Does it appear thicker or thinner than the water? Is the part containing granules of the same color as the clear part? Does this cytoplasm mix with the water? Describe the appearance of the nucleus.

B. *Movement.* Watch the moving amœba. Note the various steps in the forming of a pseudopodium. Is the movement of the animal rapid? Does it appear to move in a definite direction or at random? Do the particles in the water appear to affect its movement? Press on the cover glass with a needle point just above the amœba. How does the amœba react? Note that the movement of the amœba is produced as a result of two properties of protoplasm, *contraction* and *expansion*. A substance having these properties is said to have *contractility*.

C. In B we noted that the animal contracted and expanded without apparent cause in some cases. We noted also that under pressure it contracted more strongly. This power to respond to special stimuli is called *irritability*. Test the irritability of the protoplasm toward heat, by applying the flame of an alcohol lamp gently to the end of the glass slide. Record your observations as the heat grad-



ually increases. Other tests may be made by running solutions of various salts, etc., under the slide.

D. Feeding Habits. Examine the contents of some of the vacuoles and state your conclusions as to the form of food taken in by the protoplasm. Note and describe the method of ingulping these food particles and the forming of the vacuole. Compare several of these vacuoles as to the condition of their contents. From these observations, what do you conclude happens to food in the amœba?

(The process of taking in food is called *ingestion*. The process of dissolving ingested food is called *digestion*. The process of transforming digested food into protoplasm is called *assimilation*. This last process is evidenced by the decreasing size of the vacuole after the food is dissolved.)

E. The Removal of Wastes. Study the action of the large contractile vacuole. What does it appear to contain when expanded? Where does this substance come from? Where does it go when the vacuole is contracted? Does the vacuole pulsate regularly?

(The process of collecting the broken-down waste of the body and its removal to the outside is called *excretion*. The processes described in *D*, by means of which protoplasm is made, are spoken of collectively as *anabolism*. The processes by means of which old protoplasm is broken down and removed are spoken of collectively as *katabolism*. *Metabolism* is the simultaneous occurrence of these two actions in a living body of protoplasm.)

F. Place several amœbæ in a drop of water in a vial and cork the vial tightly. The water used should be rich in food—bacteria. Also, for comparison, make a balance preparation consisting of the same number of amœbæ mounted in the same amount of water in a watch glass, this prepara-

tion to be exposed to the air in a large vessel containing a little water to prevent evaporation. Examine at the end of a few days. What evidence have you that protoplasm requires air?

(It is the oxygen in the air that the animal uses. This property of taking in air and oxygen is part of a process called *respiration*.)

Make a list of all the properties of protoplasm as exhibited by the cytoplasm of the amœba.

XXV.—EPITHELIAL TISSUE (OPTIONAL).

Apparatus.—Prepared slide¹ of cross section of the small intestine (human preferred, but rat's or other mammal's will serve), compound microscope.

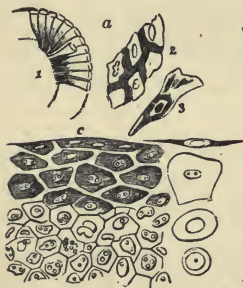


FIG. 14.
Epithelial Tissues. *a*, two forms of epithelial tissue: 1, columnar; 2 and 3, squamous; *c*, stratified tissue; *b*, simple ciliated tissue; *d*, ciliated columnar tissue.



FIG. 15.

Directions.—Focus with the high power on the cells forming the inner layer of the intestine. Draw six or eight of these cells, showing the large nucleus in each, the general outline of the cells, and the distribution of the protoplasm. Note the thinness of the cell wall and the absence of intercellular material.

¹ Prepared slides for study of tissues may be bought best of dealers, as their preparation is a matter of delicacy and skill. For those who wish to prepare their own, suitable directions will be found in standard histologies, such as Stöhr's or Schäfer's, and in Lee's "Vade Mecum."

Compare these cells (*columnar epithelium*; see Fig. 15, *d*) with those of Ex. XXII (*squamous epithelium*; see Fig. 14, *a*, 2 and 3, and *c*). How do they differ? Note the protective character of these layers of cells with reference to the underlying layers. (One feature of this protection is prevention of the action of digestive fluids upon the underlying muscles and other forms of tissue.)

XXVI.—CONNECTIVE TISSUE (OPTIONAL).

Apparatus.—Prepared slides of intermuscular tissue, cartilage, and bone, compound microscope.

Directions.—*A. Intermuscular Tissue.* Draw, under the low power. Note two classes of bundles of fibers (*white fibers* and *elastic*). The elastic fibers are single and are more sharp in outline than the white. Find one of the cells (or corpuscles) and focus with the high power. Draw it, and show in your drawing its relation to the two classes of fibers. From your study, which part of this tissue should you say was most important, the cellular part or the intercellular fibers?

B. Cartilage (hyaline). Note the solid character of the intercellular matrix, the outlines of the cells with their protoplasm and nucleus, the *lacunæ*, or pits in which the cells lie, and the capsules inclosing these lacunæ. Which part of

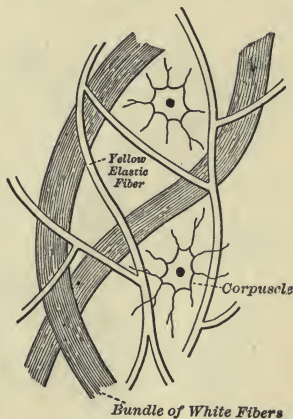


FIG. 16.—Intermuscular Tissue.

this tissue is supporting, the cells or the matrix? Draw a section, under the high power, and label all parts.



FIG. 17.—Hyaline Cartilage: *cap.*, capsule; *m.*, matrix formed by cells; *c.*, cartilage cell; *n.*, nucleus.

C. Bone. Note the matrix of spongy bone arranged in concentric rings (*lamellæ*) around the central canals (*Haversian canals*). Between the lamellæ note the irregular cavities (*lacunæ*) with their wavy branches or *canaliculi*. Note how these canaliculi connect the lacunæ with one another and with the Haversian canals.

Look in the lacunæ for the bone cells. (In ground sections of bone these will probably be wanting. They appear better in sections of de-

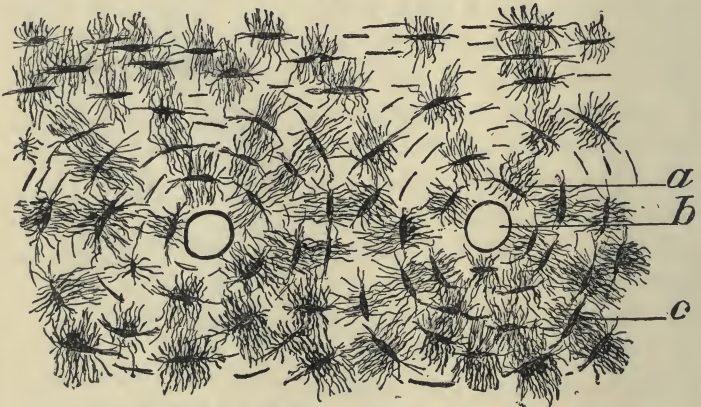


FIG. 18.—Bone: *a*, canaliculi; *b*, Haversian canal; *c*, lacuna.

calcified bone.) Draw, under high power, a section locating all the above named parts.

(Note in the three classes of connective tissue that the intercellular portion is the important part in support. The importance of the cells becomes clear when it is understood that this intercellular matrix is produced by them.)

XXVII.—MUSCULAR TISSUE (OPTIONAL).

Apparatus.—Prepared slides of striated and non-striated muscle, compound microscope.

Directions.—*A. Non-striated.* Note the long, spindle-shaped cells, the elongated nucleus, and the homogeneous protoplasm filling the whole cell. Note, further, how these cells interlace. (They are held together by a homogeneous cement substance.) Note the absence of any striation, or striping. Draw several of these cells under the high power, locating all the parts mentioned above.



FIG. 19.—A Non-striated Muscle Cell: *n*, nucleus.

B. Striated. Examine a single fiber with the high power. Note the broad, dim, transverse striæ and the narrow, light, transverse striæ. The broad stria is called *anisotropic* or *doubly refracting*, *contractile sarcoplasm*. The narrow stria is called *isotropic* or *singly refracting sarcoplasm*. Note also the more or less dim longitudinal striation. Over the whole of the fiber is stretched the transparent *sarcolemma*, or cell wall. Somewhere on the fiber may be found also several nuclei. Draw and locate all these parts of the muscle cell. (Sarcoplasm is merely another name for the protoplasm of a muscle cell.)



FIG. 20.—Portions of Striated Muscle Fibers. (The figure shows the striæ and the nuclei.)

XXVIII.—NERVOUS TISSUE (OPTIONAL).

Apparatus.—Prepared slides of ganglion cells and nerve fibers,¹ compound microscope.

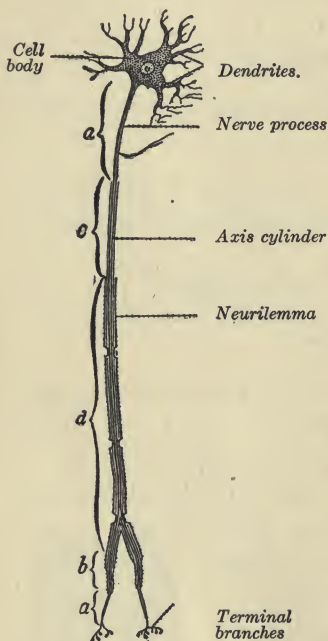


FIG. 21.—Scheme of a Neuron: *a*, free axis cylinder; *b*, axis cylinder surrounded by neurilemma alone; *c*, axis cylinder surrounded by medullary sheath alone; *d*, axis cylinder surrounded by the sheath and neurilemma and divided into segments (by constrictions called the nodes of Ranvier).

Directions.—*A. The Nerve Cell.* Note the irregular outline of the cell; the wavy projections, or *dendrites*; the rod-like projections, or *nerve processes*. Note the position of the nucleus. Has the cell one or more nerve processes? Draw and locate all parts, under the high power.

B. The Nerve Fiber. Make out from your study of the nerve fiber, the *axis cylinder* in the center. (This corresponds to the nerve process of *A*.) Next outside this is the *medullary sheath*, and on the very outside the *neurilemma*. Make a drawing showing all these parts. For their relation compare with Fig. 21.

All tissues of the body can be placed in one of the above classes—epithelial, connective, muscular, or nervous.

¹A smear preparation of spinal cord may be prepared as follows: Rub a piece of fresh spinal cord in water between two cover glasses. Mount and run under the cover glass a drop of methyl green. Both nerve fibers and nerve cells appear in such a preparation.

PRINCIPLES OF DIGESTION

XXIX.—PRINCIPLES OF OSMOSIS.

Apparatus.—Potassium bichromate, raisins, white of egg, starch, Fehling's solution, iodine solution, Millon's reagent, dialyzer. There are several forms of dialyzer described by different authors, any one of which will serve. The following form has been found very satisfactory. Obtain from the butcher some skins such as are used to hold sausage meat. Tie one of these around the base of a student lamp chimney as in Fig. 22, after cutting off the chimney so that it is only about six inches in height. Select a cork to fit tightly in the top of the chimney and, with a cork borer, puncture this to fit an eighth-inch glass tube about a foot in length. Arrange the whole apparatus as in the diagram, supporting the chimney in an outer jar so that it will not rest on the bottom. To fill the chimney, remove the cork and tube. The tube will serve as a delicate indicator of the amount of rise in the water.

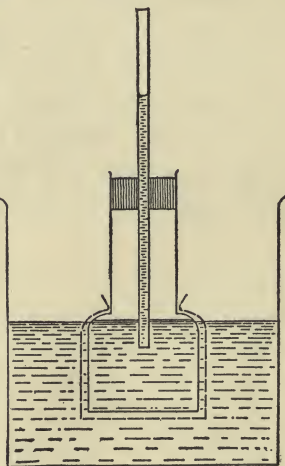


FIG. 22.

Directions.—A. Put into the dialyzer some crystals of potassium bichromate. Fill with water both the dialyzer and the outer jar until the level is the same in each. Allow them to stand for a short time. Then examine and note the level of water in the two parts. What has been the pre-

vailing direction of flow of the water? Is the color of the water in the outer jar changed? Has some of the salt solution in the dialyzer passed through the membrane? (This interchange of water and salt solution through a non-porous membrane—the sausage skin—is called *osmosis*.)

B. Chop up some raisins and place in a beaker with some water. When the grape sugar in the raisins is well dissolved, transfer this liquid to the dialyzer. Fill both dialyzer and outer jar to the same level with water as before. Note the direction of the water-flow. Test the water in the outer jar with Fehling's solution. What results? Does grape sugar in solution pass readily through the membrane? (Substances which pass readily in solution through a membrane under the above conditions may be said to dialyze.)

C. Substitute for the raisin solution a diluted starch paste. After a time note the level of the water. Record its direction of flow. Test the liquid in the outer jar with iodine solution. Does starch dialyze? Does starch crystallize like grape sugar and potassium bichromate?

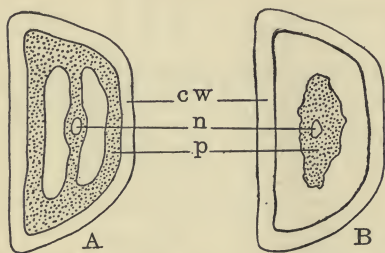
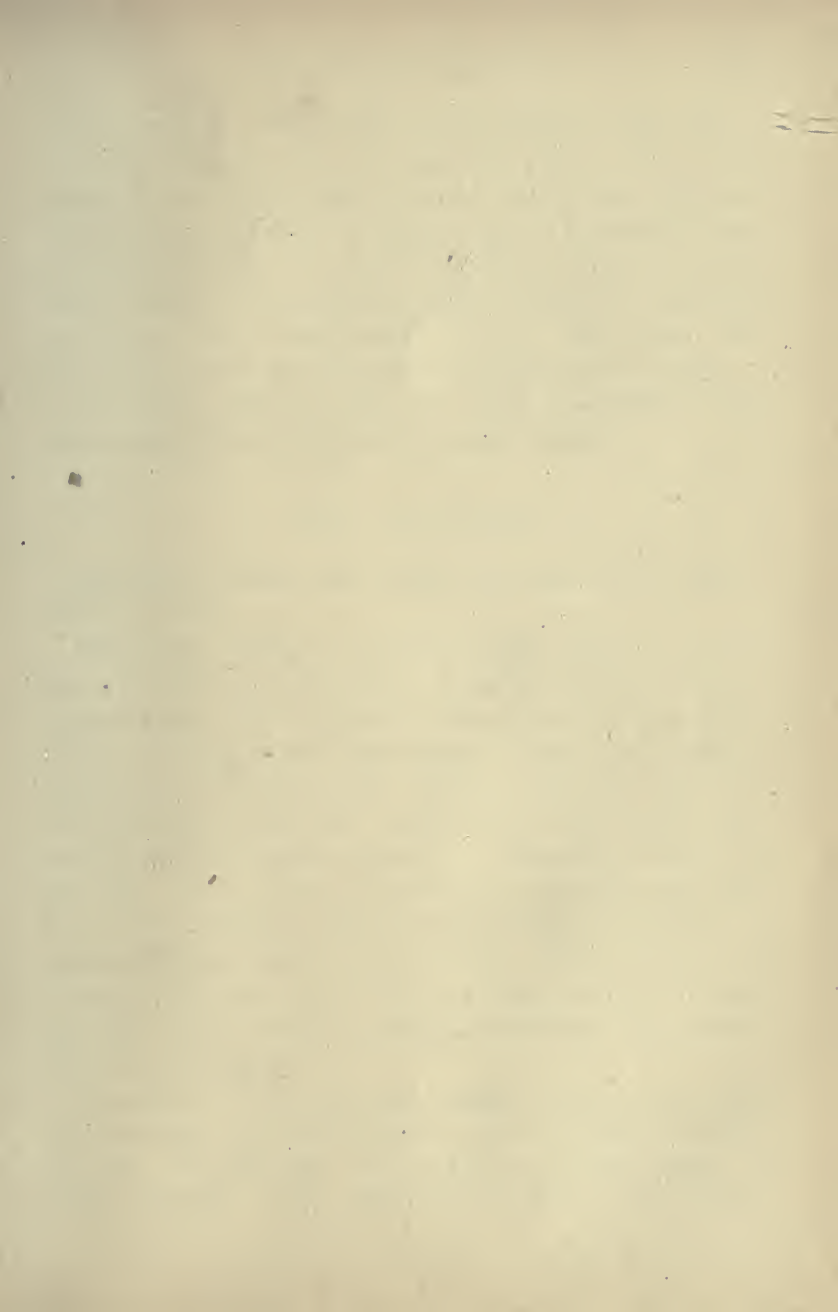


FIG. 23.—*A*, living cell; *B*, cell whose protoplasm has been killed by boiling; *cw*, cell wall; *n*, nucleus; *p*, protoplasm.

D. Substitute for the starch paste a solution made of white of egg whipped up in water. Note direction of flow of water. Test the water in the outer jar with Millon's test for proteid. Does egg albumin dialyze?

E. Cut a few slices of beet root. Wash and place a few pieces in two separate beakers. Fill each beaker half full of distilled water. Boil the slices in one



of the beakers. (This kills the protoplasm in the cells of the beets without injury to the cell walls.) Add a few drops of hydrochloric acid to each beaker, and then test with Fehling's solution for grape sugar. In which has the sugar dialyzed from the cells? In which is the water colored? Study the arrangement of protoplasm in a dead and in a living cell as illustrated in Fig. 23, and state your conclusions as to the influence of protoplasm on dialysis.

(Substances that dialyze are called *crystalloids*. Substances that do not dialyze are called *colloids*.)

XXX.—AN ENZYME.

Apparatus.—Ground malt, starch, test tubes, iodine solution, Fehling's solution.

Directions.—Make an extract of malt diastase (an *enzyme*) by shaking up five grams of ground malt with 50 c.c. of cold water. Let it stand for a few hours and then filter. Make a thin starch paste by mixing a teaspoonful of starch with a cup of boiling water. Fill two test tubes half full of this starch preparation. Test a little of the starch preparation with the iodine solution, to determine strength of reaction. Test a little of the starch preparation and also some of the diastase solution with Fehling's solution. Is grape sugar present in either of them? Now add 10 c.c. of diastase solution to one of the test tubes; heat both tubes, and keep them as near as possible at a constant temperature of 60° centigrade.

At intervals of five minutes remove a little of the contents of each tube with a pipette and test with the iodine solution. Do the same, using Fehling's solution instead of iodine solution. Is the amount of starch on the increase

or decrease in either tube? After how long a time do you get a test for grape sugar, and in which tube? Continue these tests until you get a strong test for grape sugar.

(The reason for these results is that the malt diastase—the enzyme—is slowly changing the starch into sugar. An enzyme is a substance which can bring about the transformation of one chemical compound, such as starch, into another, such as sugar, without itself being used up. The value of enzyme action in our bodies lies in the fact that by it a colloid, like starch, may be changed into a crystalloid, like sugar, which can then be absorbed through a membrane by dialysis, *e. g.*, from the stomach through the walls of the blood vessels into the blood.)

XXXI.—A FERMENT ORGANISM—YEAST.

Apparatus.—Yeast cake, molasses, eight-ounce bottle, absorbent cotton, limewater, chemical thermometer.

Directions.—Dissolve a piece of yeast cake, the size of a pea, in two tablespoonfuls of water. Pour this into the eight-ounce bottle. Add to this a tablespoonful of molasses and fill the bottle half full of water. Stopper with a plug of absorbent cotton and leave in a warm place for twenty-four hours. Record the temperature of the room in which the bottle is put, and the temperature of the mixture.

At the end of the twenty-four hours remove the stopper and examine the contents. What is the temperature? Does it smell sweet? Test the gas in the top of the bottle with a drop of lime water. What gas gives this reaction? Does the odor give you any evidence of the presence of alcohol? Examine under the low power of the compound

microscope a little of the sediment from the bottom of the bottle, mounted in water. Draw several groups of the separate elements of this sediment. (These bodies are yeast plants.)

(Yeast is a one-celled plant that, without changing its yeast character, is capable of transforming sugar into carbonic acid gas and alcohol. In its power to change a substance, without itself undergoing transformation, it acts like an enzyme and hence is called a ferment organism. Many digestive actions are performed either by enzymes or by ferment organisms, with results like that noted in Ex. XXX. Most enzymes are produced in the body by organs called *glands*.)

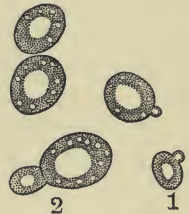


FIG. 24.—Yeast Plants: 1, a plant forming a bud; 2, the bud nearly ready to separate as a new plant.

XXXII.—STRUCTURE OF A TYPICAL GLAND.

Apparatus.—Microscope and accessories used in the study of tissues, prepared slide of crypt of Lieberkühn from the small intestine of man. (Any other gland preparation will serve.)

Directions.—Examine first with the low power. Draw the entire gland and note the following points: the kind of tissue, the arrangement of the cells, the gland *lumen*, or central cavity. With the high power examine a few of the cells and their contents. Draw and



FIG. 25.—A Salivary Gland: a, lumen of a gland in longitudinal section; b, a gland in cross section; c, connective tissue.

note the position of

the nucleus, the protoplasm, and the secretion in various cells. Fig. 26 illustrates the relation of the simple, tubular gland, such as you have just studied, to the compound forms.



FIG. 26.—Forms of Glands.

ORGANS AND PROCESSES OF DIGESTION

XXXIII.—DISSECTION OF A RAT'S DIGESTIVE ORGANS.

Apparatus.—Chloroformed rat,¹ dissecting tray with wax lining, scissors, forceps, bristle probes, 10% alcohol or 1% formalin.

Directions.—Lay the rat on its back in the tray, stretch, and tie or pin the legs as in the diagram. Cover with 10% alcohol or 1% formalin.

Locate the lower end of the breast bone and slit the skin from this point to the anus. On each side at the middle point of the slit, make a slit at right angles. Turn back the four flaps and pin them.

Note the thin membrane (*peritoneum*) lining the abdomen. Is it flexible? Remove this and, without disturbing the underlying parts, locate the stomach, the liver, and the coiled intestine.

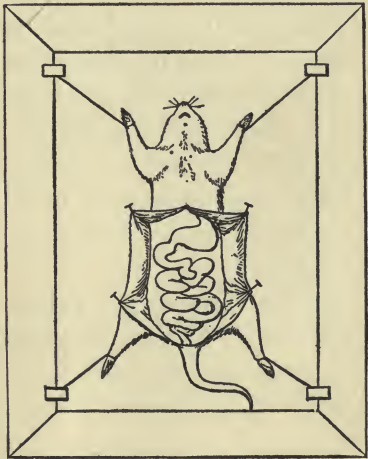


FIG. 27.

Press the intestine downward and determine the size, position, shape, and color of the stomach. Find the ends

¹ This exercise may be made a demonstration. In that case a larger animal such as the rabbit would be preferable.

that are connected with the intestine (*pyloric* end) and with the esophagus, or gullet (*cardiac* end). Note the covering of blood vessels.

In the fold of the intestine (*duodenum*) next to the stomach, locate the fatty-looking *pancreas*. Find its *duct* and trace its connection with the duodenum.

Press forward the liver and, on its posterior surface, find the *bile sac*. Locate the connection of this with the two lobes of the liver (the *hepatic ducts*). Open this sac and, with the probe, find its connection (the *bile duct*) with the pancreatic duct and the duodenum. Note that the bile duct and pancreatic duct fuse and enter the duodenum by a common duct.

Examine the membrane (*mesentery*) which supports the intestine. Note its blood vessels. Carefully unravel the intestine (Caution! do not break it) from the stomach to the anus. Determine the relative lengths of the small and the large intestine and the method of their joining. (This connection is guarded by a valve which acts in such a way as to prevent matter returning from the large to the small intestine.)

Slit the stomach just below the gullet entrance and, with the probe, find its connection with the mouth. Above the liver and the stomach, find the muscular partition (*diaphragm*) separating the abdominal from the thoracic cavity.

Illustrate, by a diagrammatic drawing, the connections of the following parts: mouth, gullet, stomach, liver, pancreas, small intestine, large intestine.

Carefully remove the stomach, liver, pancreas, and intestines, and preserve the rest of the animal for further dissection in 85% alcohol or 4% formalin.

XXXIV.—THE TEETH.

Apparatus.—A hand mirror, a molar tooth sawed in vertical sections, an apple.

Directions.—*A. Kinds of Teeth.* With the aid of the mirror and the finger count the number of teeth on each jaw. Is the number the same? Note that they may be divided into four classes according to shape. How many broad

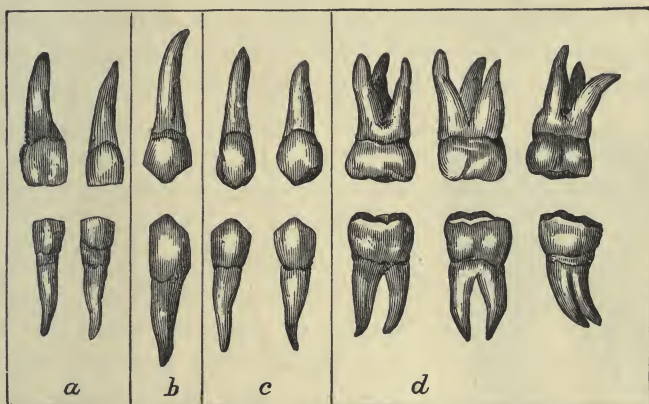


FIG. 28.—*a*, incisors; *b*, canines; *c*, premolars; *d*, molars.

teeth (*incisors*) have you in the front of each jaw? How many with one point on the surface (*canines*)? How many with two surface points (bicuspid or *premolars*)? With more than two surface points (*molars*)? Tabulate these numbers as follows:

	UPPER JAW	LOWER JAW
Incisors		
Canines		
Premolars		
Molars		

Grand Total

Examine the mouths of animals, such as the squirrel or rat, the cat or dog, and the horse or cow. How do they differ as to the kind and number of their teeth? What kind of food does each animal eat? Which kind of food requires the most chewing? Do you see any connection between the food and the kind of teeth which predominates in each animal?

B. Structure of a Tooth. Draw a section of a molar tooth. Find the following parts: the crown, the neck, roots or fangs, the covering of the crown (*enamel*), the covering of the fangs (*cement*), the central or *pulp cavity* with nerve and blood

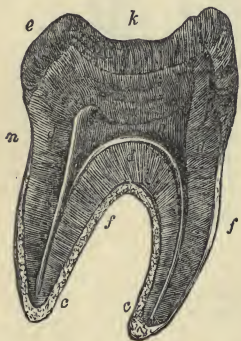


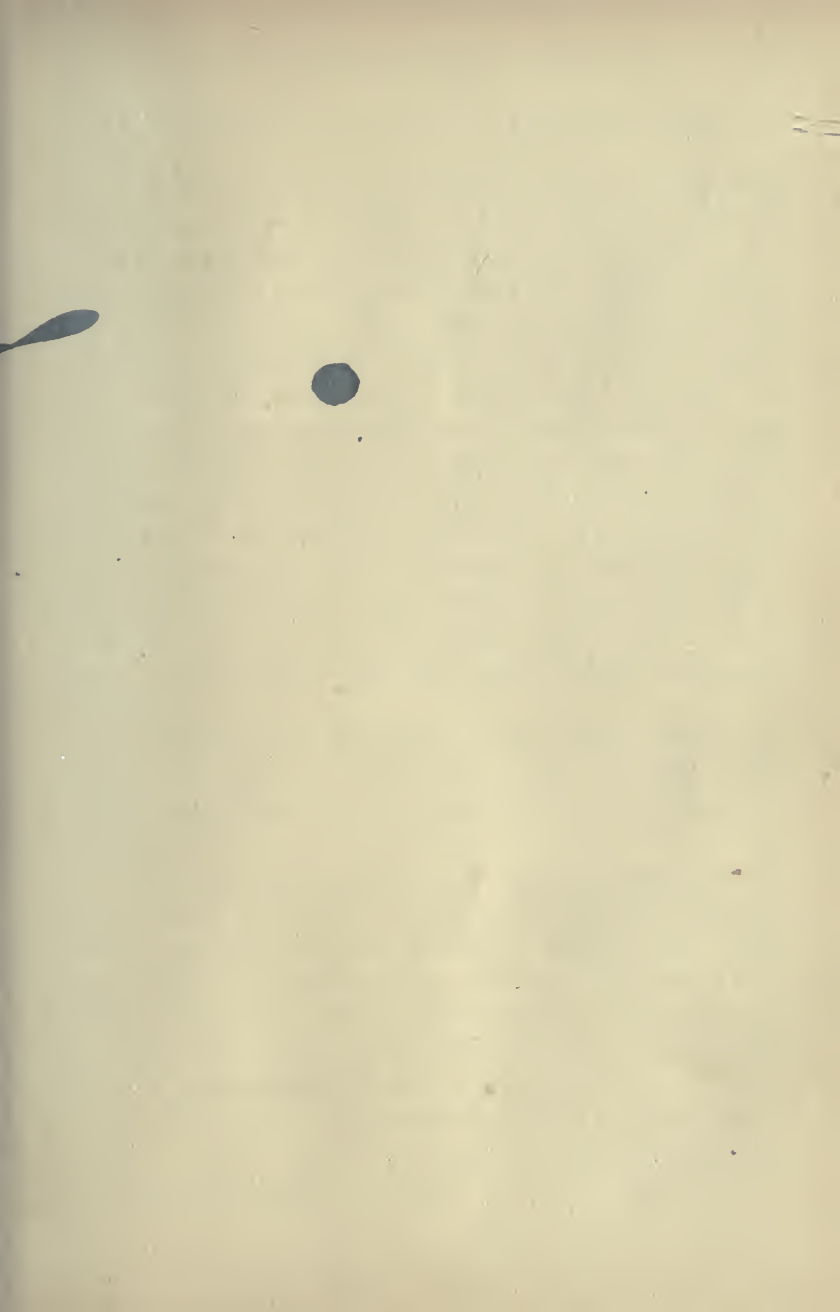
FIG. 29.—A Molar: *k*, crown; *n*, neck; *f*, fangs; *e*, enamel; *d*, dentine inclosing the pulp cavity; *c*, cement.

vessel aperture, the middle layer (*dentine*). Label all these parts in your drawing. Examine, if possible, the jaw of a human skeleton to show the insertion of the teeth in it.

C. The Use of the Teeth. Bite off a piece of apple and chew it. Answer the following questions: Which teeth are used in the biting off of the apple? Which to chew it into

small pieces? Why are these latter best adapted to break up the food? Of what advantage is it that a horse's molars are ridged on the surface? Could you tell from the examination of the teeth the kind of food an animal eats?

When a tooth decays what part actually decays? What is the difference in the functions of the enamel and of the dentine? How does the location of the nerves in the pulp cavity protect them? Why is a decayed tooth apt to ache?



XXXV.—PREPARATION OF DIGESTIVE FLUIDS (OPTIONAL).

A. Collection of Saliva. Wash the mouth out thoroughly with warm water to remove all foreign matter. Bow the head forward, turn out the lower lip, and collect the clear saliva as it flows over the center of the lip. Collection should be made only a short time previous to use in experimenting. The saliva should give no reaction with Fehling's solution.

B. Artificial Gastric Juice. Obtain a pig's stomach. Cut it open and wash its contents out by gently flushing it with water. Remove the mucous membrane from the cardiac end, and after drying this with filter paper mince it and bottle with four or five ounces of glycerine. (The glycerine dissolves the pepsin.) After three days filter through muslin. The filtered solution may be kept indefinitely. When required for use add 0.2% hydrochloric acid¹ in the ratio of one part of the acid to ten parts of glycerine solution.

A substitute for the above is solid pepsin powder dissolved in water. For use, this should be treated with 0.2% hydrochloric in the same way as the glycerine solution.

C. Artificial Pancreatic Juice. Soak the pancreas of a pig in water over night. Then remove it from the water, mince, and add ten times its volume of glycerine. (Glycerine dissolves the pancreatin.) Filter as with gastric juice. This preparation is suited to the digestion of starches and proteids. For action on fats add ten volumes of 1.5% solution of sodium carbonate, shake well, and filter.

A substitute for the glycerine solution may be made by

¹That is, dilute hydrochloric acid containing 2 parts of the concentrated commercial acid to 998 parts of water.

dissolving the solid pancreatin powder in water. For fats add to this the sodium carbonate solution in the same way as to the glycerine solution.

D. Bile. Open and extract the contents of an ox gall or dissolve prepared ox gall in water.

E. Temperature Conditions. To obtain the best results in all artificial digestion experiments keep the materials used as near a constant temperature as is possible. For this purpose it is suggested that a constant temperature water bath be used, if possible. If this is unavailable an ordinary drying oven may be used with an Argand burner. Another substitute is a double boiler,—such as is used in cookery,—with the Argand burner. Place in test tubes the fluids and materials to be digested. Cover the inner chamber of the double boiler with a wooden cover having holes bored to fit the test tubes, and suspend the tubes in these. Heat the water in the outer part to the temperature desired and adjust the Argand burner to maintain just that temperature.

XXXVI.—DIGESTION OF THE MOUTH—SALIVA.

Apparatus.—A little salt, dry cracker, dilute starch paste, white of egg, olive oil, saliva, litmus paper, Fehling's solution, concentrated hydrochloric acid, test tubes, constant temperature apparatus.

Directions.—*A. General Functions of Saliva.* Clear the mouth of saliva by swallowing, and wipe dry the top of the tongue. Place on the tongue a bit of salt. Can you taste the salt? Close the mouth, letting the salt stay on the tongue. What happens in the mouth? Where does the saliva come from in the mouth? Where is it made? Was the presence of the salt on the tongue sufficient to cause its

flow? What does it do to the salt? Can you taste the salt now? Do you think the effect would be the same if the salt had been dissolved in water? Verify by placing a drop of salt water on the dry tongue. Name two functions of saliva that this experiment shows.

Again clear the mouth of saliva, wipe the tongue dry, and place on it some powdered cracker. Try to swallow the cracker. Is it easily done? With the tongue moisten the cracker with saliva and try to swallow. Is swallowing easy now? What is another function of saliva?

Chew some of the cracker slowly and note if any change takes place in its taste. Place on the dry tongue some cracker moistened with water. Is the taste the same? What power has the saliva that is not due to its liquid quality only? (This last power of the saliva is called its chemical power as distinguished from its purely mechanical properties.)

B. Enzyme Action of Saliva. Place in four test tubes a little thin starch paste. Add a cubic centimeter of clear saliva to each, and label Tubes 1, 2, 3, 4. Add a few drops of concentrated hydrochloric acid to the fourth tube. In a fifth tube place 1 c.c. of saliva and a little minced white of egg, and label Tube 5. In a sixth tube place 1 c.c. of saliva and a few drops of olive oil. Label Tube 6. Shake each tube. Pack Tube 2 in ice, and keep Tube 3 in boiling water.

Tube 1. Test the mixture with litmus paper. Is it acid or alkaline? Now heat gently to a temperature of 36°C . Keep at this temperature for twenty minutes and then test with Fehling's solution. What has the saliva done to the starch? See Ex. XXX. What caused the change in the taste of the cracker in A?

Tube 2. After the second tube has been in ice twenty minutes test with Fehling's solution. What is the effect of cold on the action of saliva?

Tube 3. Keep the third tube in boiling water twenty minutes and test as above. What is the effect of high temperatures on the action of saliva?

Tube 4. Heat the fourth tube to 36°C . for twenty minutes and then test as above. Does the Fehling's solution give any test for sugar? (Acids prevent the enzyme action of saliva.)

Tube 5. Heat the fifth tube to 36°C . for twenty minutes and then test with Fehling's solution. Does saliva convert white of egg to sugar? (Saliva does not affect any proteid.)

Tube 6. Treat Tube 6 in the same way as Tube 5. Does saliva convert olive oil to sugar? (Saliva does not affect any fats and oils.)

(The action of saliva is due to the presence of an enzyme called *ptyalin*, which converts starch to grape sugar.)

Tabulate all the functions of saliva.

XXXVII.—DIGESTION OF THE STOMACH—GASTRIC JUICE.

Apparatus.—Glycerine solution of pepsin or solid pepsin dissolved in water, 0.2% hydrochloric acid, concentrated hydrochloric acid, caustic soda, alcohol, minced white of egg, starch, olive oil, milk, rennet dissolved in water, test tubes, dialyzer, constant temperature apparatus, distilled water, materials for xanthoproteic and biuret tests.

Directions.—*A. Action of the Enzyme Pepsin and Hydrochloric Acid.* Label seven test tubes Tube 1, Tube 2,

etc., and prepare them as follows: In the first place 5 c.c. of the glycerine solution or dissolved pepsin and dilute with 10 c.c. of water. In the second tube put 15 c.c. of the 0.2% hydrochloric acid. In the third, fourth, and fifth tubes place 15 c.c. of glycerine solution which has been diluted previously with ten parts of 0.2% hydrochloric acid to one of glycerine solution. Prepare the sixth tube in the same way as the third and then add 5 c.c. of concentrated hydrochloric acid. Prepare the seventh tube in the same way as the third and then add 5 c.c. of caustic soda solution. Add to each of the seven tubes some minced white of egg, and shake. Place Tubes 1, 2, 3, 6, and 7 in a temperature of 36° C. and keep at this temperature for twenty-four hours. Place Tube 4 in ice. Keep Tube 5 in boiling water.

At the end of twenty-four hours examine all the tubes. In which is the white of egg dissolved? What nutrient forms the bulk of white of egg? From the result in Tube 2, does hydrochloric acid alone dissolve white of egg? What is the effect of cold on the action? of high temperature? Does excess of hydrochloric acid help or hinder action? Why is a basic substance like sodium bicarbonate given in case of sour stomach? Does pepsin act in the presence of a strong base like caustic soda? (Pepsin requires for its action a slightly acid medium, such as is found in the stomach.) Will ptyalin act in an acid medium? Can it convert starch to sugar in the stomach?

Now place the contents of Tube 3, containing the dissolved proteid, in the dialyzer, and put distilled water in the outer jar. After twenty-four hours test the water in the outer jar by the xanthoproteic and biuret tests for proteid. Does the proteid solution pass through the membrane? Does white of egg dialyze? (See Ex.

XXIX.) This form of crystalloidal proteid is called *peptone*.

Add a little alcohol to some of the peptone solution remaining inside the dialyzer. Filter off the precipitate. Does it resemble coagulated albumin? Add water to some. Does it dissolve? Does coagulated albumin dissolve?

Prepare two tubes in the same way as Tube 3, but substitute starch paste in one, and olive oil in the other, for the white of egg. Examine after twenty-four hours. Are they dissolved?

B. Action of the Enzyme Rennin. Add a little of the rennet ferment (which contains rennin) dissolved in water, to a little milk in a test tube. What happens to the milk? Filter off the precipitate. Test it for proteid. (Rennin coagulates the proteid in milk.)

Now add a little of the glycerine solution of gastric juice to some milk. Does the action resemble that of the rennin solution? (Gastric juice contains rennin. Pepsin will not convert milk proteid into peptone unless the milk proteid is previously coagulated; hence the necessity of the rennin action.)

XXXVIII.—DIGESTION OF THE INTESTINE—PANCREATIN AND BILE (OPTIONAL).

Apparatus.—Prepared solution of pancreatin (see Ex. XXXV), 1.5% sodium carbonate solution, solution of ox gall, hydrochloric acid, caustic soda, materials for food tests, constant temperature apparatus.

Directions.—*A. Action of the Proteid Enzyme of Pancreatin (Trypsin).* Prepare five tubes as follows: Into each

put 10 c.c. of solution of pancreatin, and some minced white of egg. Label Tubes 1, 2, 3, 4, and 5. To Tube 4 add 5 c.c. of hydrochloric acid and to Tube 5 add 5 c.c. of caustic soda solution. Keep Tubes 1, 4, and 5 at 36° C., Tube 2 on ice, and Tube 3 in boiling water for twenty-four hours.

At the end of twenty-four hours examine all the tubes and note in which the proteid is dissolved. What is the effect of cold on the action? of high temperatures? of excess of acid? of excess of alkali? Test Tube 1 with litmus. What is its reaction?

(Pancreatin contains an enzyme called *trypsin*, which, like pepsin, acts on proteid and converts it into peptone.) From the above experiments, does trypsin require an acid medium? (Pepsin will not act in the intestine because the contents of the intestine are alkaline.)

B. Action of the Starch Enzyme of Pancreatin (Amylopsin). Place a little pancreatin solution in three test tubes and label Tubes 1, 2, and 3.

Tube 1. Test with Fehling's solution. Does the pancreatin solution contain grape sugar?

Tubes 2 and 3. Add to the second and third tubes a little dilute starch paste. Test with litmus. Is the solution acid or alkaline? To Tube 3 add enough hydrochloric acid to give a distinct acid reaction with litmus. Keep both tubes at 36° C. for twenty-four hours. Then test with Fehling's solution. Record results. Are they the same in both tubes?

(Amylopsin will act only in a basic medium.)

C. Emulsion and Action of Fat Enzyme (Steapsin). To pancreatin solution add the 1.5% sodium carbonate solution as directed in Ex. XXXV, C. Shake some olive oil in

a test tube with this solution. Let it stand a moment. Do the oil drops reunite after separation as when shaken with water? Is the oil dissolved? (This is an *emulsion*, which is formed when separated particles of oil are kept from reuniting by some surrounding medium. Such action is called mechanical as contrasted with the chemical action of a ferment.)

Boil some olive oil with caustic soda. Smell this solution and describe its odor. Taste it. What has been formed? Is this product soluble in water? (The conversion of fat into soluble soap is called *saponification*. This action is performed by the pancreatin, without boiling, by aid of the contained ferment called *steapsin*.)

D. Action of Bile. Examine some of the gall from an ox gall. What is its color? taste? (Human gall, or bile, is golden brown.) Test some of this ox gall with litmus. Is it acid or alkaline? Shake up some of the gall with olive oil. Does it emulsify the oil? Add some of the gall (bile) to minced white of egg and keep at 36° C. for twenty-four hours. At the end of that time test the mixture with food tests. Has the bile converted the white of egg? Repeat, using starch paste instead of white of egg, and follow with food tests. Has the bile converted the starch? (Bile contains no enzyme and hence can not convert any form of nutrient.)

XXXIX.—DIGESTION OF MINERAL SALTS (OPTIONAL).

Apparatus.—Phosphate of lime, sodium chloride (common salt), dilute hydrochloric acid (10%), test tubes, evaporating dish.

Directions.—Shake up some sodium chloride with water in a test tube. Do the same with some phosphate of lime

in another tube. Do the salts dissolve? Soluble salts may be digested in all parts of the digestive tube, since all contain water. Salts which are insoluble require special treatment.

Place some phosphate of lime in the 10% hydrochloric acid. What happens? Is this solution? To determine this point pour some of the lime and acid mixture into an evaporating dish and evaporate to dryness. Does the residue taste like phosphate of lime? Is it soluble in water? Since the hydrochloric acid is used up, the action is not that of an enzyme. The resulting residue is not phosphate of lime; hence the action is not a simple solution. What has taken place is a chemical combination of hydrochloric acid and the phosphate, producing a salt which is soluble in water. This process illustrates a method of converting insoluble salts into forms soluble in water, and is a process that takes place in the stomach.

XL.—TABULATION OF NUTRIENT DIGESTION (OPTIONAL).

Directions.—Fill out the following table from the results obtained in the preceding exercises. If a given nutrient is digested by more than one reagent, indicate by separate entries for each as indicated in the table.

NUTRIENT.	REGION OF ALIMENTARY TRACT DIGESTED IN.	DIGESTIVE REAGENT.	NAME OF DIGESTED PRODUCT.
Proteid			
Proteid			
Starch			
Starch			
Fats			
Fats			
Soluble Salts			
Insol. Salts			

XLI.—MICROSCOPIC ANATOMY OF THE DIGESTIVE TRACT (OPTIONAL).

Apparatus.—Prepared slides of the cross sections of the walls of the esophagus (middle part), stomach (pyloric section), small intestine (injected blood vessels); compound microscope.

Directions.—Make drawings of each section studied and label the parts. See Figs. 30 and 31.



FIG. 30.—Vertical Section of the Coats of the Stomach: *d*, surface of mucous membrane, and mouths of gastric follicles; *m*, gastric tubuli, or follicles; *mm*, dense connective tissue; *sm*, sub-mucous tissue; *cm*, transverse muscular fibers; *lm*, longitudinal muscular fibers; *s*, fibrous, or serous, coat.



FIG. 31.—Section of Injected Small Intestine of Cat: *a*, *b*, mucosa; *g*, villi; *i*, their absorbent vessels; *h*, simple follicles; *c*, muscularis mucosæ; *d*, sub-mucosa; *e*, *e'*, circular and longitudinal layers of muscle; *f*, fibrous coat. All the dark lines represent blood vessels filled with an injection mass.

BLOOD

XLII.—GENERAL PROPERTIES OF BLOOD.

Apparatus.—Glass slides and cover glasses, magnifier, microscope, needle, normal salt solution (0.6% solution), neutral carminate of ammonia.

Directions.—Wind a handkerchief tightly around the thumb, just below the joint. Now bend the upper joint. The blood will collect on the top of the thumb just below the nail. Sterilize a needle by holding it a second in a flame, and prick the thumb just below the nail. The blood from the puncture may be easily and quickly transferred to a glass slide.

A. With a magnifier examine a drop mounted as above. Is it all liquid? Is it the same color throughout? Describe the color at the edge of the drop. Let the drop remain on the slide for ten minutes and examine again. Is it liquid now? Prick at it with the needle point and describe its consistency. This formation is called a *clot*. Examine the puncture on the thumb with the magnifier. Has it stopped bleeding? What is the condition of the blood on the surface of the puncture? Does it resemble the condition of the drop on the slide? Bind up the thumb as before and flex the upper joint. Does the puncture bleed again? Wash off the clot with water. Does the bleeding begin again now? What is the advantage of this clotting action of the blood when exposed to air?

B. Mount a drop of blood quickly, and examine at once with the high power of the microscope. Note the rouleaux of *colored corpuscles*. What is their color? Note also the *white* or *colorless corpuscles* (colorless corpuscles tend to stick

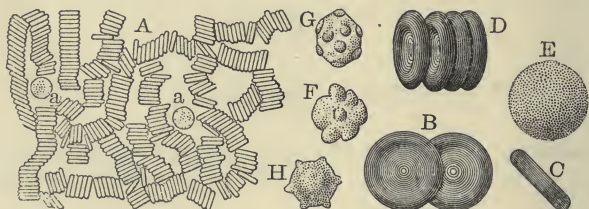
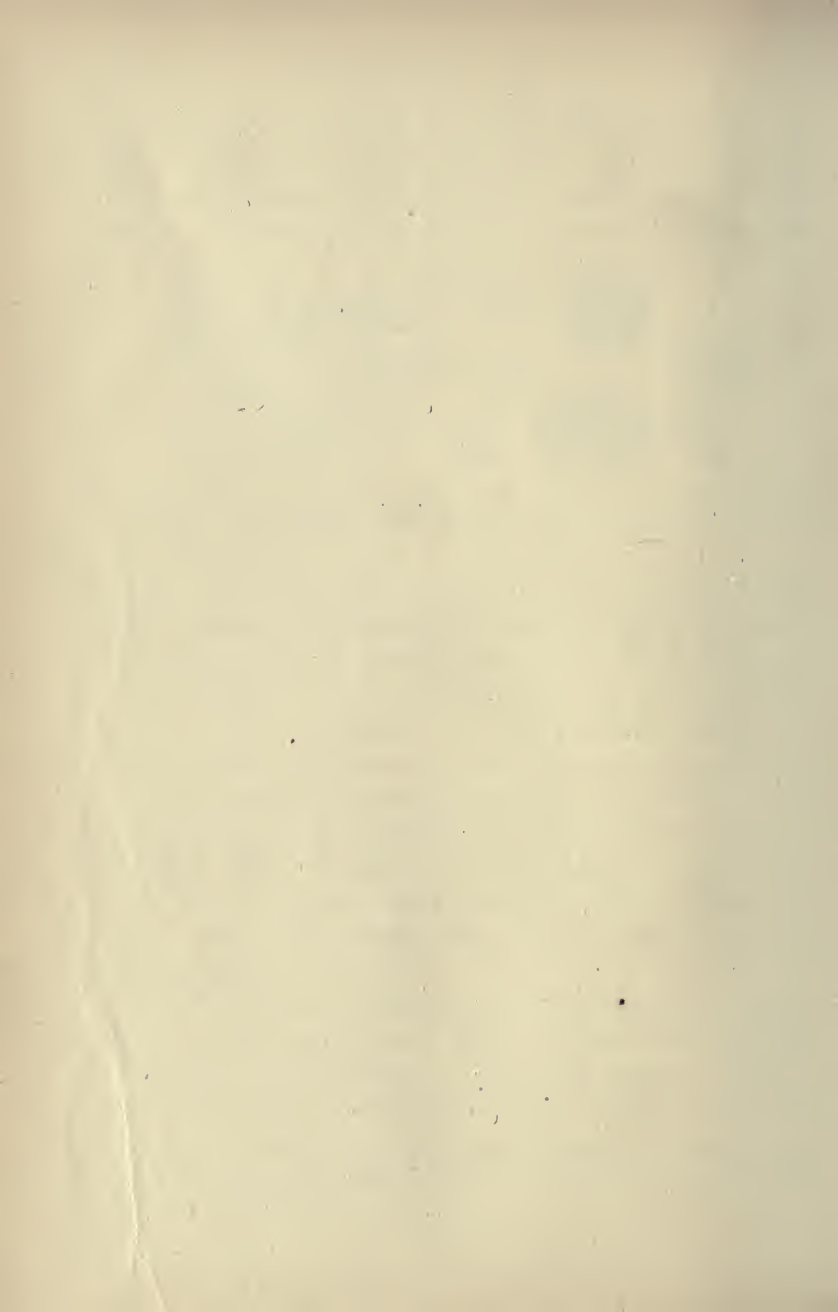


FIG. 32.—Blood Corpuscles: *A*, red corpuscles in *rouleaux*; *a, a*, colorless corpuscles ($\times 400$); *B*, red corpuscles in focus; *C*, view of edge; *D*, three-quarters view; *E*, red corpuscle swollen with water; *F, G, H*, distorted red corpuscles.

to glass; hence they will remain if the cover glass is pressed with a needle so that the current will drive the others aside; and they can then be more readily seen). What is the color of the liquid in which the corpuscles are floating? This liquid is called the *plasma*. Let this preparation stand for fifteen minutes and then run under the cover glass a drop of strong solution of neutral carminate of ammonia.¹ This decolorizes the red corpuscles but brings out the nuclei of the white corpuscles and the fibrin filaments. Draw some of the white corpuscles and note the shape of the *fibrin filaments*. Note how the entanglement of these filaments forms the foundation of the clot.

C. Mount a drop of blood as in *B*, but before covering it with the cover glass, add a drop of normal salt solution. This causes the separation of the red corpuscles. Draw a surface view and an edge view of a red corpuscle under the

¹A permanent mount may be made of this preparation if a little glycerine is allowed to diffuse under the cover glass and the cover slip is then cemented to the glass with gold size.



high power. How do red corpuscles differ in appearance from the white corpuscles? Have they a nucleus?

XLIII.—STUDY OF BEEF OR PIG BLOOD.

Apparatus.—Five-ounce bottles, fresh blood, egg beater, test tubes, food-testing materials, constant temperature apparatus, compound microscope, slides and cover glasses, distilled water, dialyzer.

Directions.—A quart or more of fresh-drawn blood should first be obtained from a butcher. Divide this among the five-ounce bottles as follows:

Bottle 1. Fill with fresh blood and cork so as to exclude all air.

Bottle 2. Fill two-thirds full and leave uncorked.

Bottles 3, 4, and 5. Fill two-thirds full and cork.

Place the remainder of the blood in a basin and whip vigorously with an egg-beater or twigs. Take off the stringy substance that collects on the beater, and wash it in water until it has lost its red color. Put it in Bottle 6 and add to it a little water.

Pour the whipped blood into a suitable-sized bottle and label it Bottle 7. Leave uncorked.

A. Study of Coagulation or Clotting. Place Bottles 1 and 2 in ordinary room temperature. Examine frequently for several days. In which bottle does the clot form quickest? Does the absence of air in Bottle 1 have any effect on the rate of clotting?

Place Bottle 3 in a constant temperature of 36° C. and pack Bottle 4 in ice. In which does the clot form quickest? Does temperature affect the rate of clotting?

Place Bottle 5 under the same conditions as 1 and 2 but

shake from time to time. Does this affect the rate of clotting?

Place Bottle 7 with 1, 2, and 5. Examine after three days. Has this blood clotted? What is missing in it? (The substance is called *fibrin*.)

Summarize the conditions best suited to clotting. The exact reason why blood clots when it is not in a healthy blood vessel is unknown.

B. Study of the Clot. Pour off the liquid from all the bottles in which a clot has formed and place it in Bottle 8. (This liquid is called *serum*.) Then break one of the bottles containing a clot and remove the clot entire. What is its shape? color? consistency? Cut off a thin slice of it and examine it under the microscope. What parts can you distinguish? Does it contain any corpuscles? The jelly-like substance is to be found in its pure state in Bottle 6. Examine some of this fibrin. What is its color? Test it for proteid. What is the result? Explain in a few words the formation of a clot and the part played in its formation by the fibrin and the corpuscles.

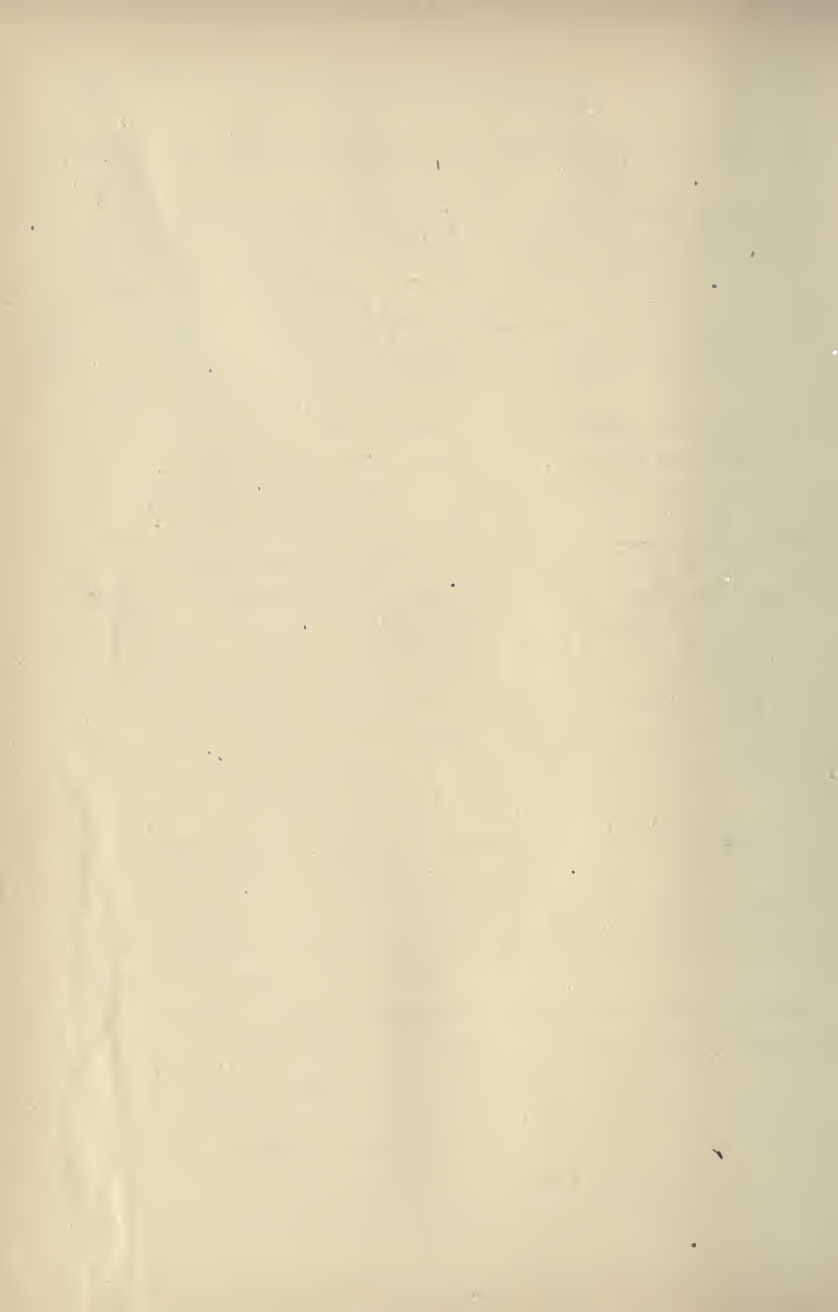
C. Study of the Serum. Examine the liquid in Bottle 8. What is its color? Why is it not red?

Test a little with iodine solution for starch. Since starch must be digested before it can be absorbed into blood, why should you expect this result?

Test some of the serum with Fehling's solution for the presence of grape sugar. Do you get a strong test? What does this result suggest as to the amount present?

Burn a little serum on a piece of platinum foil. Does it contain any mineral matter?

Place a drop on a piece of unglazed paper and let it evaporate. Does it leave a grease spot?



Heat a little serum and test for proteid. Can the proteid present be fibrin? Reasons? What use is made of the foods present in serum? How do they get into the serum? (See Ex. XXX.) What is one function of the blood?

D. Study of Defibrinated Blood. Examine the contents of Bottle 7. How does this blood differ from fresh blood? from serum?

Place some of this blood in the dialyzer. Fill the outer jar with distilled water. Does the color of the water in the outer jar change? After a time test the water in the outer jar for proteid, grape sugar, minerals. What part of the blood dialyzes?

Fill a bottle half full of defibrinated blood and shake it vigorously. Does it change in color? What was mixed with the blood by shaking the bottle? (One function of the colored corpuscles is to take up oxygen. This function, which heightens their color, is due to the iron they contain; see Ex. VI.) Look up in your text the use of the white corpuscles.

CIRCULATION AND THE BLOOD SYSTEM

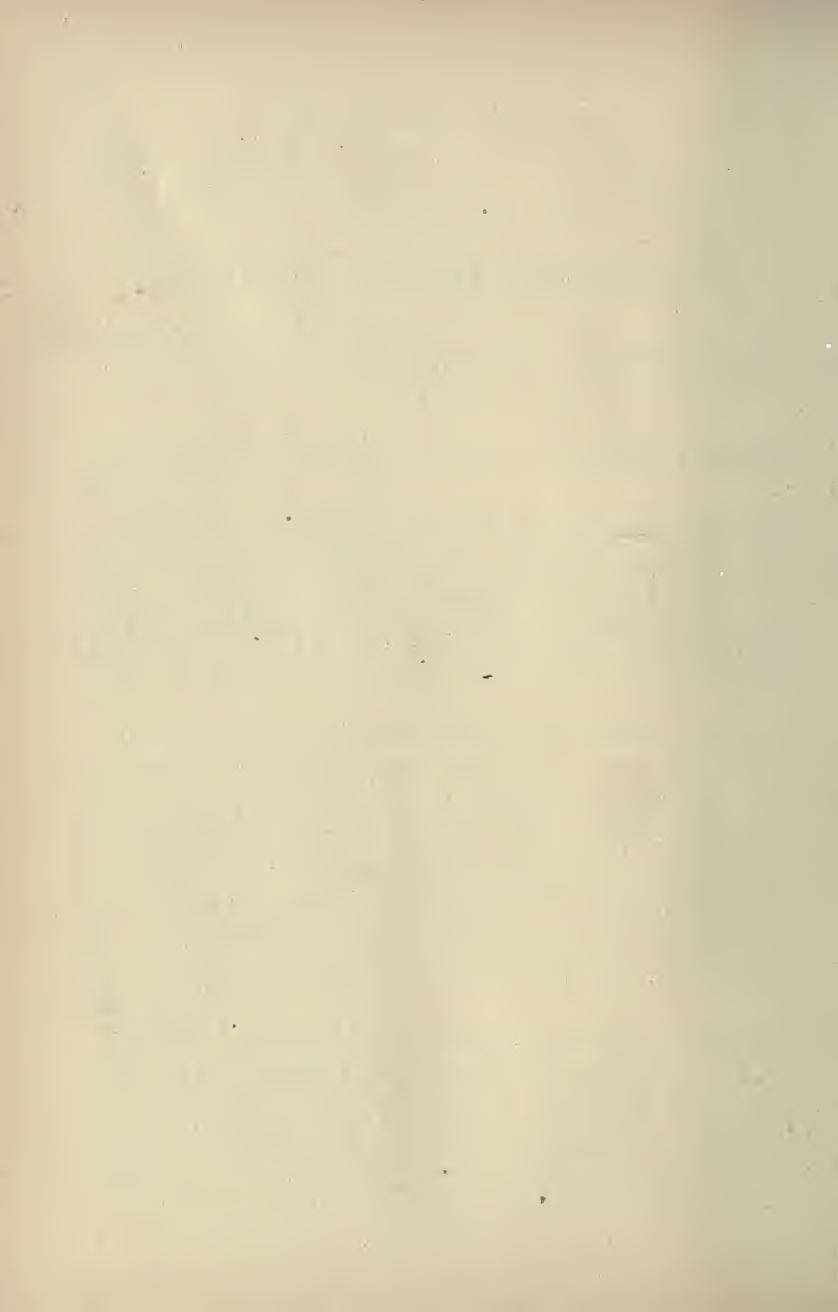
XLIV.—PROPERTIES AND LOCATION OF ARTERIES AND VEINS.

Apparatus.—A watch with a second hand, a needle, a chemical thermometer.

Directions.—Examine the back of the hand and wrist and locate the dark-colored veins. Is the blood this color? Place your finger on a vein. Can you feel any motion? Is there any difference in the size and prominence of the veins when you exercise violently? Why should you expect this result?

Find your pulse on the palm side of the wrist. Count its beats and record the number per minute. Test this rate at various times of the day. Is it uniform at all times? Test your body temperature at the same time by placing the bulb of the chemical thermometer under the tongue. Does the temperature vary with the pulse rate? Does either increase after violent exercise? If food is burned up by exercise, and blood contains oxygen and food, how do you account for these effects?

Examine other parts of the body for veins and arteries (pulse always indicates the presence of an artery). Which are most numerous on the surface? Which are best protected? The bleeding of a cut artery is much more difficult to stop than that of a vein, owing to its pulsation.



Examine the skin on the back of the hand between two veins. Can you see any blood vessels? Place the finger on this part. Can you feel any pulse? Prick through the skin at this point with a sterilized needle. Does the puncture bleed by spurts or steadily? The small blood vessels filling these places are called *capillaries* on account of their small size (*capillus*=a hair). They connect the veins and arteries.

XLV.—CIRCULATION IN A FROG'S FOOT.

Apparatus.—Compound microscope, cover slip, live frog, shingle, wet absorbent cotton, and cloth.

Directions.—Bind a live frog in wet absorbent cotton, leaving one leg extended. Fasten the frog, so bound in place, on a frog board (a piece of shingle with a hole the size of a cover slip at one end). Stretch the web of the foot over the hole in the board. Fasten it securely, with the stretched web as level as possible. Mount this board on the microscope stage in such a way as to bring the web-covered hole under the objective of the microscope. With a pipette place a drop of water on the top of the web, and cover with a piece of cover slip. Illuminate in the usual way and focus first with the low and then with the high power.

Note the network of blood vessels and the slow-moving stream of corpuscles within them. Are the corpuscles the same size and shape as those in the human blood? Is there more than one kind? Observe that in some of the blood vessels the blood moves in spurts at regular intervals. What kind of vessels are these? Does the blood in these flow from or toward the body? Follow the course of the blood from these into the smaller tubes where the corpuscles move in

almost single file. Do these show pulsations? Trace the flow from these into larger vessels where no pulsation is evident.



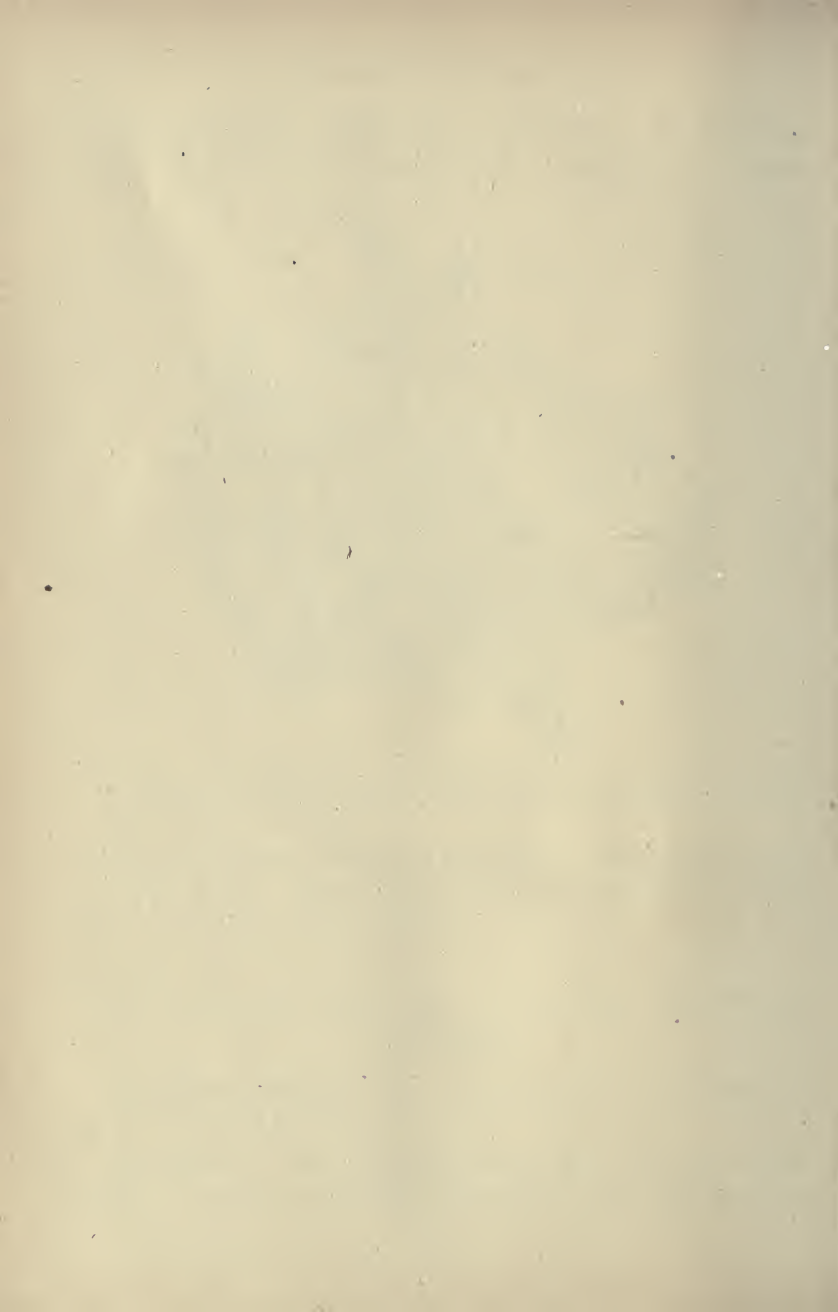
FIG. 33.—Capillary Circulation in the Web of a Frog's Foot, $\times 100$: *a*, *b*, small veins, *d*, capillaries in which the corpuscles are seen to follow one another in single series; *c*, pigment cells in the skin.

Note the direction of flow in these tubes. What is the name of these tubes? Define artery, vein, and capillary in terms of the direction of blood flow.

XLVI.—MINUTE STRUCTURE OF ARTERIES AND VEINS (OPTIONAL).

Apparatus.—Prepared slides of cross sections of arteries and veins, compound microscope.

Directions. Note that both artery and vein have three coats: a lining of epithelial cells called here *endothelium*, a



middle layer consisting of a mixture of muscle and elastic fibers, and the outside layer or coat of connective tissue bundles. Make careful drawings of the two preparations, showing the location and form of these layers, and label the above-mentioned parts. In which of the two forms of blood vessels is the elastic and muscular coat thickest? Why should you expect this condition from the method of flow of blood in each? What is the special advantage of the elastic fibers in the artery? In what way do they aid to keep the capillaries filled at the end of an artery pulsation? Is the pressure greatest in arteries or in veins?

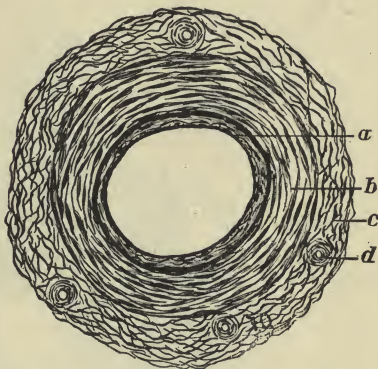


FIG. 34.—Cross Section of an Artery: *a*, endothelium; *b*, muscular layer; *c*, connective tissue; *d*, small artery to nourish large one.

XLVII.—STRUCTURE OF THE HEART.

Apparatus.—Sheep's heart from the butcher with pericardium attached, bristle seekers, dissecting instruments.

Directions.—Locate the parts named below, and make drawings to show their position.

A. Note that the heart moves easily inside a loose sac. Cut this *pericardium* open and observe its slippery inner coat. Note a similar coat on the outside of the heart. What lies between these two coats? This liquid and the slippery coats prevent friction when the heart pulses.

B. Carefully cut away the pericardium from the blood vessels, and the fat from the surface of the heart. Locate

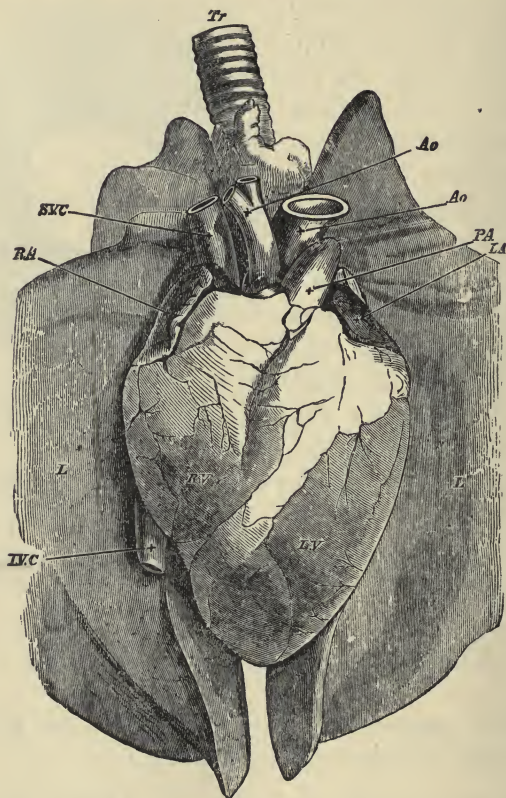
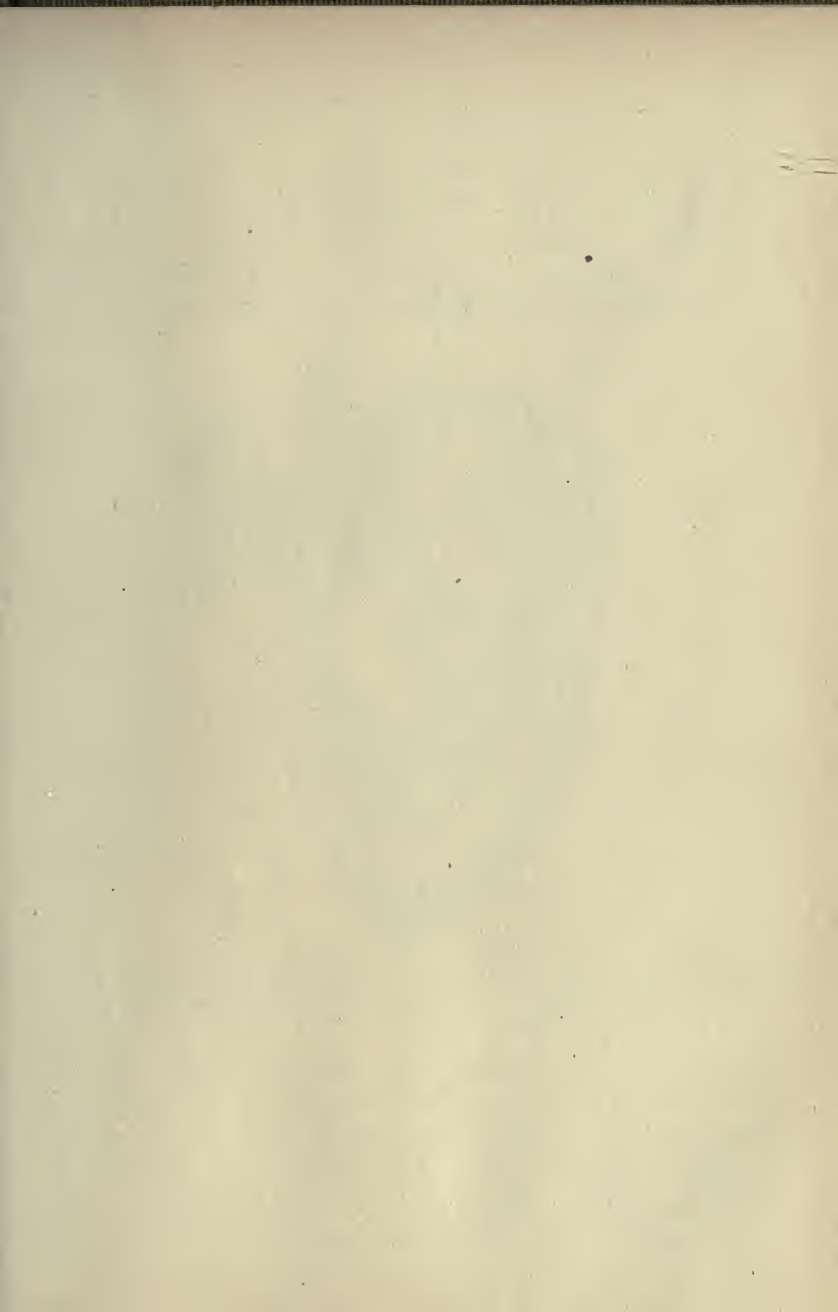


FIG. 35.—Heart in position with pericardium removed (Human): *Tr*, trachea; *L*, lungs; *RA*, *LA*, right and left auricles; *RV*, *LV*, right and left ventricles; *Ao*, aorta (two branches); *SVC*, *IVC*, superior and inferior venæ cavæ; *PA*, pulmonary artery.

the *aorta*, *venæ cavæ*, *pulmonary veins* and *artery*; and push bristle seekers through these blood vessels into the heart.

C. Examine the outside of the heart and locate the follow-



ing parts of the heart proper: *right* and *left auricles*, *right* and *left ventricles*. By right and left are meant the parts of the heart that are right and left in its position in the body. Which parts have the thickest walls? The walls are made of muscle, and these thick-walled parts do the pumping.

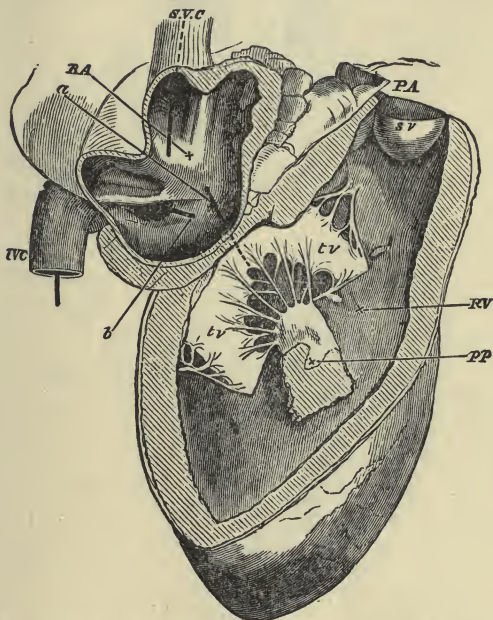


FIG. 36.—Right Auricle and Ventricle (Sheep): *RA*, *RV*, right auricle and ventricle; *IVC*, *SVC*, inferior and superior venæ cavæ; *a*, *b*, bristle seekers showing connections between auricle and ventricle, auricle and vena cava; *PA*, pulmonary artery; *tv*, tricuspid valve; *pp*, papillary muscle; *sv*, semilunar valves.

D. Cut off carefully the front walls of the right auricle and ventricle. By means of the bristles locate the entrance into the auricle of the *inferior* and *superior venæ cavæ*, and the entrance into the ventricle of the *pulmonary artery*. Find the connection between the auricle and the ventricle and note the *tricuspid valve* that closes this entrance. Lo-

cate also the *chordæ tendinæ* that attach this valve to the *papillary muscles* on the surface of the heart. What is the effect of the contraction of the ventricle on the action of this valve?

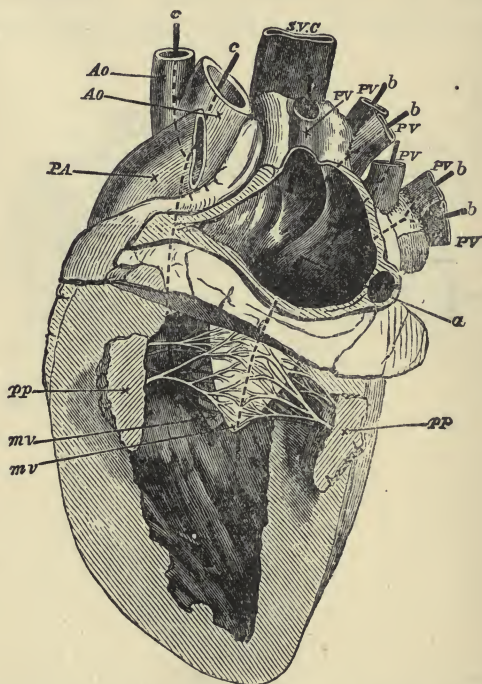


FIG. 37.—Left Auricle and Ventricle (Sheep): *a, b, c*, bristle seekers showing connections of auricle with ventricle, of auricle with veins, and of ventricle with arteries; *PV*, pulmonary veins; *pp*, papillary muscles; *mv*, mitral valve; *PA*, pulmonary artery; *ao*, aorta; *SVC*, superior vena cava.

Note finally the *semilunar valves* at the entrance to the pulmonary artery. How does their arrangement prevent the backward flow of blood into the heart?

E. Cut off the front walls of the left auricle and ventricle in the same way. Have they any connection with the right

side of the heart? Locate, with the aid of the bristles, the entrance of the *pulmonary veins*. How many enter the auricle? Find the entrance from the auricle to the ventricle, and the *mitral valve* which guards this entrance. Does it show chordæ tendinæ and papillary muscle attachments? How does it differ in shape from the tricuspid? Locate the *semilunar valves* at the entrance of the aorta.

Make a careful diagram of the course of circulation through the heart to the lungs and back to the heart and body.

THE BODY SKELETON

XLVIII.—STUDY OF THE SKELETON.

Apparatus.—Human skeleton.

Directions.—Tabulate as follows, the various classes of bones:

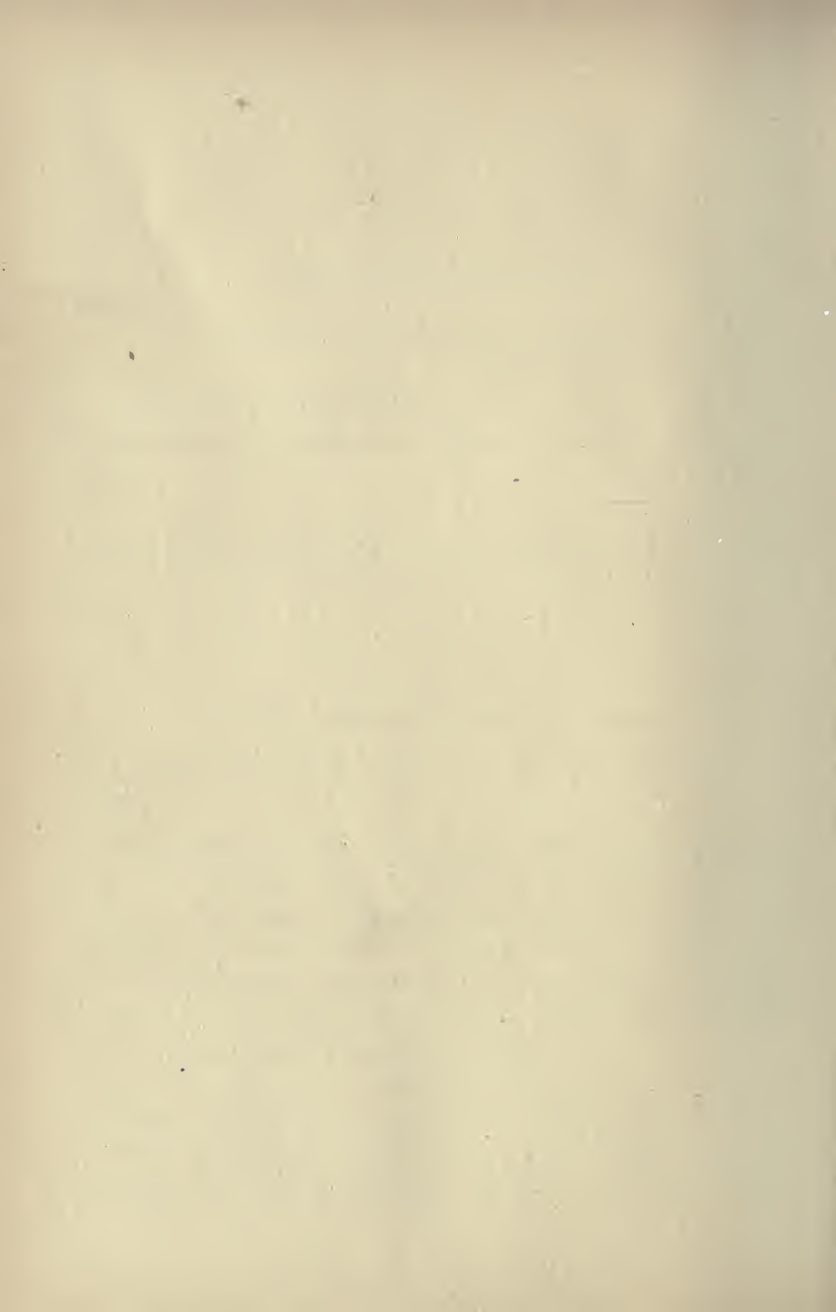
KIND OF BONE.	No.	NAME OF BONE.	LOCATION IN BODY.	FUNCTION.

XLIX.—GROSS STRUCTURE OF BONES.

Apparatus.—Fresh rib, thigh bone, and dorsal vertebra; saw, needle.

Directions.—A. *The Rib*, a flat bone. Draw the bone from the flat side. What is found at the ends of the bone? What is the color, consistency, and function of this substance? Bend the bone. Is it flexible? Pick off the membrane (*periosteum*) that covers the bone. Does it separate easily from the bone? Does it tear easily? Are all parts of the bone protected by this covering?

Saw the rib across. Examine the section and draw it, labelling the parts in the order in which they occur. What part is periosteum? hard bone? spongy bone? marrow? Examine the central marrow. What is its color? How



does it feel? Heat some in water in a tube. What collects on the top of the water?



FIG. 38.—Thigh Bone, in Longitudinal Section.

B. The Thigh Bone, or Shank, a long bone. Draw the bone, and shade with different colors the parts that are covered with cartilage and with periosteum. What is the function of the enlarged heads of this bone? Of what advantage is it that they are irregular in surface?

Saw the bone lengthwise, draw, and label the parts. In what portion of the bone is the marrow most plentiful? Is the shaft solid? What is the advantage of this condition?

C. The Dorsal Vertebra. Draw a dorsal vertebra from the side and from the top. With the aid of the diagram locate the following parts: The body of the vertebra, spinous process, transverse processes, spinal cavity, rib articulations,

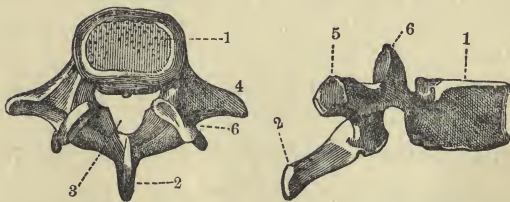


FIG. 39.—A Dorsal Vertebra: 1, centrum or body; 2, spinous process; 3, spinal cavity; 4, transverse process; 5, rib articulation; 6, vertebral articulation.

vertebral articulations. How are the articulations protected? What is the function of the processes?

L.—COMPOSITION OF BONE (OPTIONAL).

Apparatus.—Two clean ribs, a soup bone split in two, 20% hydrochloric acid, bottle big enough to hold rib, evaporating dish, food-testing materials, Bunsen burner.

Directions.—A. Place one of the ribs in the bottle and fill the bottle with the 20% hydrochloric acid. Let it stand for a few days. At the end of that time examine it. Has it changed in shape? Take it out of the bottle and bend it. What power has it lost? What substance is left? Hold a little of it in the flame. Does it burn? Pour a little of the acid from the bottle into the evaporating dish and evaporate to dryness. What kind of substance is left? What material did the acid dissolve out of the bone?

B. Burn the other rib. What is the shape of the part that is left? Is it flexible? Put some of it in the acid. Does it dissolve? Name the two main constituents of bone.

C. Cover the split soup bone with water and gradually bring to a boil. Strain off the liquid and let it cool. What do you find floating on the surface? What forms as it cools? What is the character of this substance? Test for foods.

LI.—STRUCTURE OF A JOINT.

Apparatus.—Fresh leg joint of lamb or veal, scalpel.

Directions.—Examine the tissue that binds the two bones together. What is the character of these bands, or *ligaments*? Are they flexible? How do they control the direction of movement of the bones? Cut off the ligaments with a scalpel. Note the liquid found within. What does it look like? (It is a lubricant called *synovial fluid*).

Examine the ends of the bones. With what are they covered? Press this surface. Is it elastic? What is the advantage of this? Is the surface smooth? Of what ad-

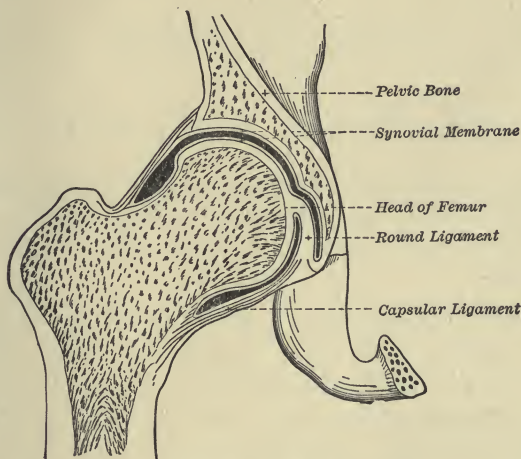


FIG. 40.—A Joint.

vantage is this? What is the reason for the enlarged ends of the bones? for their irregular surfaces?

LII.—FORMS OF JOINTS.

Apparatus.—The human skeleton.

Directions.—Examine the following joints and describe the range of motion of each: Knee, elbow, vertebral, shoulder, hip, jaw, head and spine, bones of the skull, ribs.

Name the bones united in each case and classify the joints under the following names: Hinge, ball and socket, gliding, rotary, dovetail, symphysis.

Which of the above are movable joints? fixed?

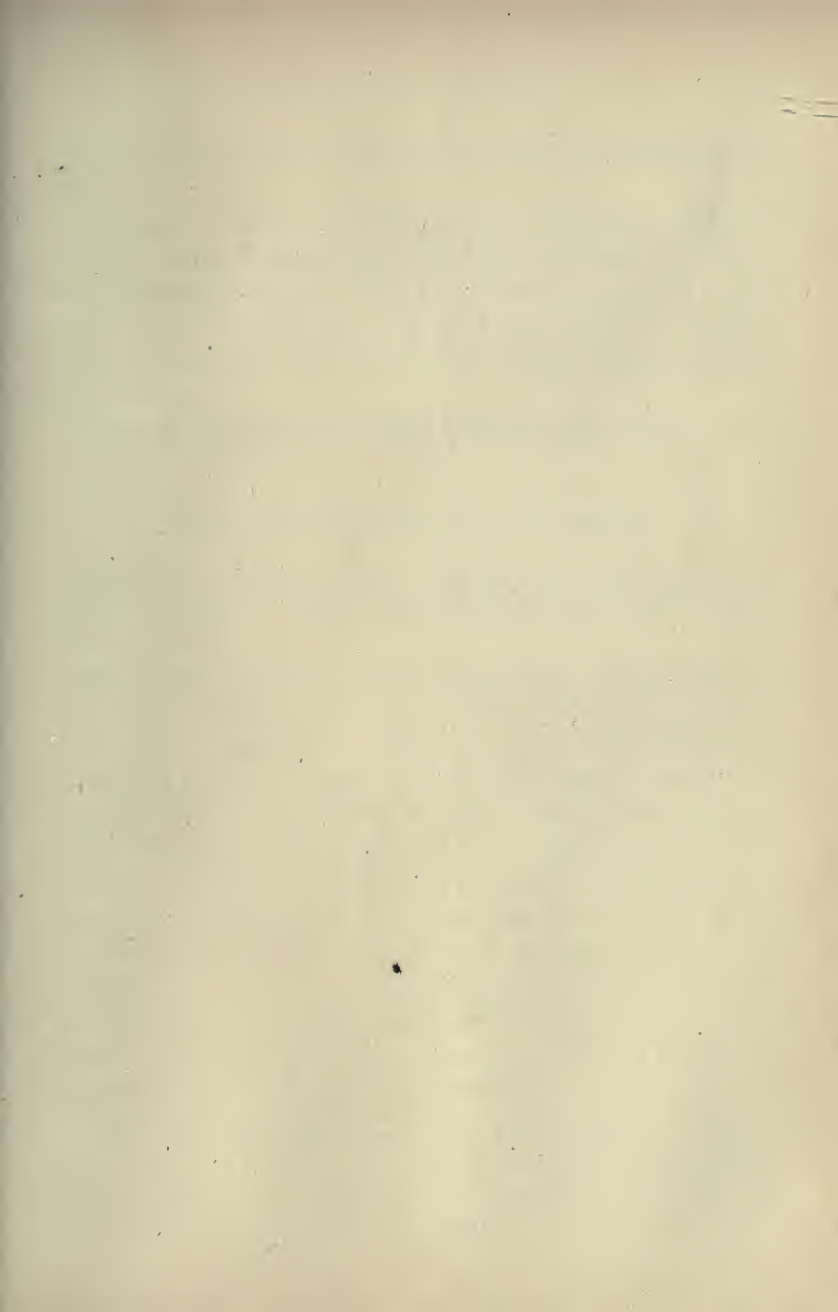
MUSCLES AND MOTION

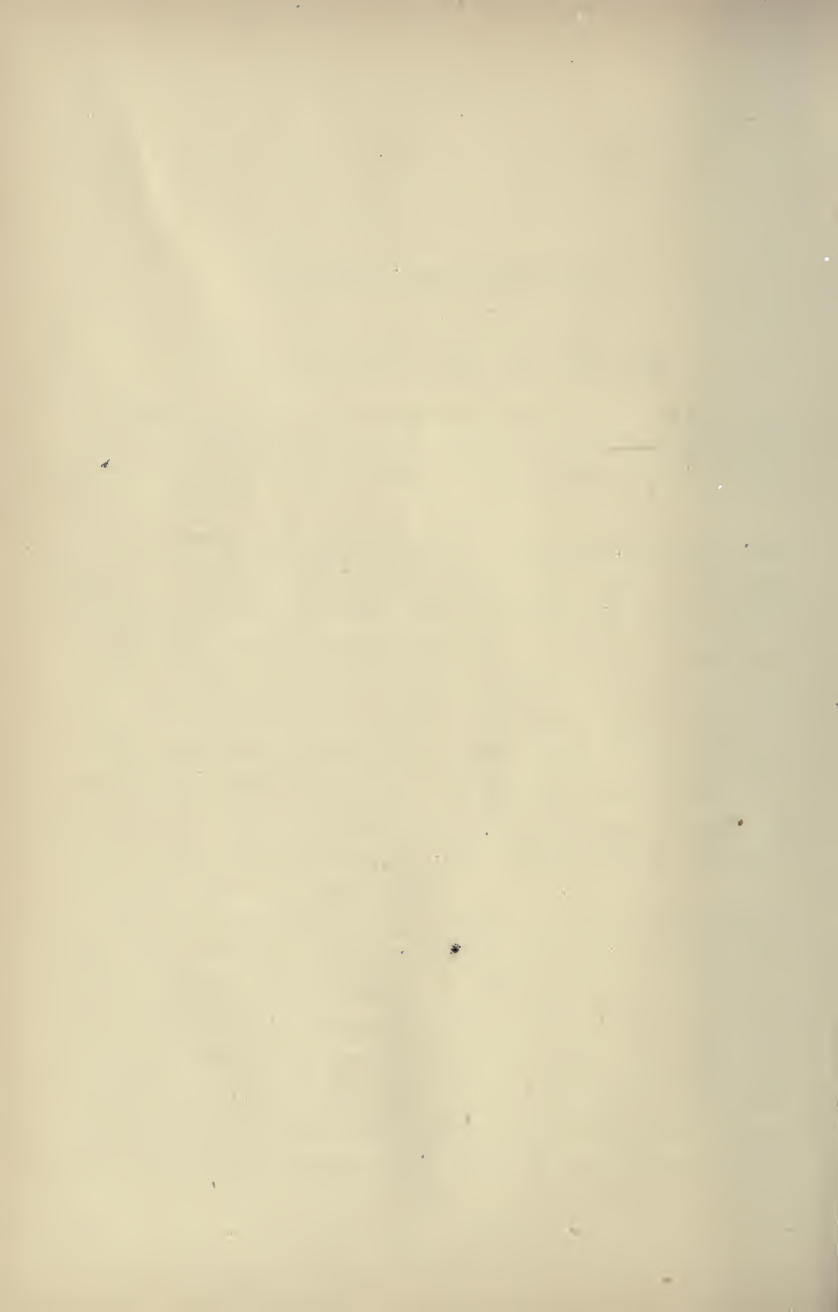
LIII.—DISSECTION OF THE MUSCLES.

Apparatus.—The body of the rat used in Ex. XXXIII (any other animal will serve the purpose and if a demonstration is desired for the study of the leg muscles the leg of a sheep may be substituted), scalpel.

Directions.—Carefully cut off the hind leg of the rat, close to the hip joint, and remove the skin. Note the muscles covering the bones and the glistening white muscle sheath (*perimysium*) covering each muscle. At the ends of the muscles note the white *tendons*. Are the muscles attached directly to the bones? The end of the muscle that moves most in contraction is called its *insertion*; the one that moves least, its *origin*. Where are the tendons most numerous? How does this arrangement avoid clumsiness in the foot? Compare with the arrangement in your own hand and foot. Is it the same?

Separate the tendons and muscles without cutting them, and pull on each to determine what part of the leg it controls. Muscles that extend a joint are called *extensors*, those that bend it are called *flexors*. Note that all these muscles have a thick center, or belly, and tapering ends with tendons attached at the ends. Those muscles with two tendons at the origin are called *biceps*; those with three, *triceps*. Examine one of these tendons. How is it different from a





muscle? Is it elastic? Why should you expect this from its use?

Remove the skin from the sides of the body. How do the underlying muscles differ from the leg muscles? Have they tendinous ends? What two classes of muscles based upon their form can you name from your study? Mention some other parts of the body where these kinds of muscles can be found.

Preserve the rest of the rat's body for future use.

LIV.—GROSS STRUCTURE OF MUSCLE.

Apparatus.—A bellied muscle from the rat or frog (a piece of fresh beef will serve), needles, compound microscope and slides, food-testing materials.

Directions.—Boil the muscle in water for a few moments and pick it to pieces with the needles. Note that it separates easily into bundles. Why is cooked beef more easily chewed than raw? Examine the perimysium covering the bundles. What sort of tissue is it? Describe its appearance. What purpose does it serve? Place one of these bundles in a drop of water on a slide and with the needles tear off the perimysium and tease the bundle into fibers. Examine one of these fibers under the low power of the microscope. Note its covering (*sarcolemma*) and its striated appearance. All muscles under direct nerve control (*voluntary muscles*) show this striation. (For the minute anatomy of this fiber see Ex. XXVII.)

Apply the xanthoproteic and other food tests to pieces of the muscle. From the strength of the various reactions, what is the main constituent of muscle? Why does an athlete require a diet rich in proteid?

LV.—NERVE MUSCLE PREPARATION (OPTIONAL).

Apparatus.—Put a frog in a bottle or jar, pour in a little chloroform, and cork the bottle. As soon as the frog is still, remove it from the jar and, with a scalpel, sever the spinal cord just back of the skull. With a wire, destroy the brain and spinal cord. Dissect



FIG. 41.—*sc*, sciatic nerve; *g*, gastrocnemius; *ad*, *b*, etc., other muscles.

away a hind leg; remove all the muscles except the gastrocnemius, and separate this at its lower attachment. Fasten the femur strongly in a clamp. With a pointed glass rod separate the sciatic nerve at the upper part; do not touch it with metal instruments. Into the lower end of the muscle insert a hook and connect it with a lever as in Fig. 42. Connect a copper wire, insulated except at the end which is to be used as an electrode, with each pole of a battery of two dry cells. For convenience a key of some kind may be inserted in the circuit to make and break.

Directions.—Touch the free end of the nerve with the two electrodes. What happens to the muscle? Record the extent of the action. This shows that nerve stimulation may cause the muscle to move. Keeping the electrodes in contact with the nerve, note whether the action continues. Remove the electrodes. What happens? Repeat this process several times and mark the distance that the lever moves each time. Is it the same? Does the action increase or decrease? This result illustrates what may happen from overstimulation; namely, *muscle fatigue*.

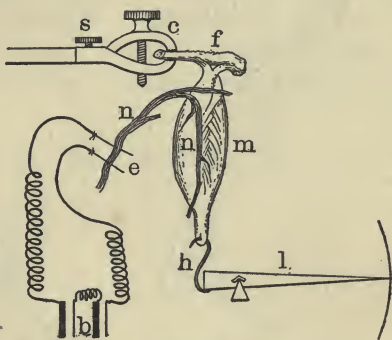


FIG. 42.—Nerve Muscle Preparation: *s*, set screw; *c*, clamp; *f*, femur; *m*, gastrocnemius; *n*, sciatic nerve; *h*, hook; *l*, lever; *e*, electrodes; *b*, battery.

Repeat the experiment, applying the current to the body of the muscle instead of the nerve. Compare with the results of the first experiment as to extent and strength of the action.

In both of the above experiments what property of the muscle is stimulated? Why is muscle called contractile tissue?

LVI.—STUDY OF LEVER ACTION (OPTIONAL).

Apparatus.—Wooden bar with holes near the ends and at the middle (exactly halfway between the end holes), spring balances.

Directions.—A. Support the bar by the middle hole (see Fig. 43, A), and trim the bar till it balances level. Fasten

the spring balances in the two end holes. Pull down on each, keeping the bar horizontal. Compare the pulls registered by the balances. What is their relation? Attach one balance halfway between the end and middle holes, keeping the second balance in the other end hole. Pull until the bar

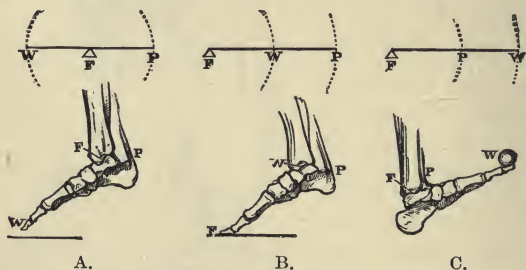


FIG. 43.—Forms of Levers: *A*, 1st class; *B*, 2d class; *C*, 3d class; *W*, weight; *F*, fulcrum or pivot; *P*, pull.

is level as before. What is the relation of the registered pulls now? Verify the following law by changing the position of the two balances.

Weight \times perpendicular distance from the pivot equals
Pull \times perpendicular distance from the pivot. (Perpendicular distance is measured from the pivot at right angles to the direction in which the force is acting.)

This arrangement of lever is called a lever of the first class.

B. Support the bar by one end hole, and at the extreme end attach a weight so that the bar will balance level; then insert the balances in the other two holes (see Fig. 43, *B*). Pull down with the one nearest the pivot (Weight), and up with the one at the end (Pull). Record the pull and weight when the bar is level, measure the distances from the pivot, and see if the law of *A* still holds. This arrangement is called a lever of the second class.

C. If the pull nearest the pivot be called the Pull and the

other the Weight, the arrangement is called a lever of the third class (see Fig. 43, *C*).

LVII.—LEVERS OF THE BODY (OPTIONAL).

Directions.—*A.* Locate on the upper arm the biceps muscle, or flexor of the arm. Where is it attached to the forearm and how far (perpendicular distance) from the elbow? Measure the perpendicular distance from the elbow to the center of the palm. If now we put a weight of ten pounds in the palm and bend the arm, what class of levers is illustrated? How much force is required on the part of the muscle to raise ten pounds' weight? By selecting different weights to lift, determine the maximum strength of the biceps muscle. What muscle is used in striking an outward blow with the fist? Where is it located and inserted? Note that the flexors and extensors in other parts of the body are usually arranged in pairs.

B. Examine the relation of the muscle, weight, and pivot in the following cases, and tell which class of lever each illustrates: Jaw action in chewing, flexing of the fingers, movement of the legs in kicking, bending the body, movement of the foot about the ankle (see Fig. 43).

NOTE.—The instructor can suggest other problems of the above nature to make clear the laws of lever action.

RESPIRATION

LVIII.—DISSECTION OF A RAT'S LUNGS.

Apparatus.—Body of the rat used in Exs. XXXIII and LIII, scalpel, glass tube of one-eighth inch diameter.

Directions.—Remove the skin from the surface of the ribs and throat. Examine carefully the muscles between the ribs (*intercostals*). Seize the base of the breast bone and move it up and down. Notice the motion of the *intercostals* during this process.

Insert the glass tube in the top of the windpipe through the throat opening, and blow gently through this tube. Observe the motion of the ribs and the motion of the muscular *diaphragm* that forms the partition between the abdominal and thoracic cavities. Press the diaphragm up with the finger and note that air is forced out of the tube.

Now cut the ribs where they join the breast bone, and press them back to expose the organs of the cavity. Sketch the position of the lungs and heart. Compare with Fig. 35, page 76. Note the texture of the lungs and observe the windpipe (*trachea*) with its cartilage rings. (These are necessary to prevent collapse of the tube.) How is the windpipe connected with the lungs?

Carefully dissect out the lungs and windpipe and float them in water. Cut them at the entrance of the windpipe and trace out the *bronchi* and their branches. How do these

branches end? (This large amount of branching allows the air to be brought in contact with very many small blood vessels, through the walls of which oxygen is absorbed by the blood.)

LIX.—MECHANICS OF RESPIRATION.

Apparatus.—A glass bell jar open at the top, a glass tube with a toy balloon firmly bound to one end, a stick with a knob, a piece of sheet rubber, a one-holed stopper to fit top of bell jar.

Directions.—Pass the tube through the stopper and seal it in place with wax. Insert the stopper in the top of the bell jar with the balloon inside the jar. Tie the knob into the center of the rubber sheet and fasten the latter tightly across the base of the bell jar, leaving the stick outside to serve as a handle. With this arrangement the tube corresponds to the trachea, the balloon to the lungs, the rubber sheet to the diaphragm, and the jar to the thoracic cavity.

Now move the handle downward so as to stretch the diaphragm. What happens to the balloon? What causes this action? Move the handle upward. What happens to the balloon now? Why? How does the diaphragm secure rhythmic inhaling and exhaling, *i. e.*, inflow and outflow of air?.

LX.—STUDY OF EXPIRED AIR.

Apparatus.—Chemical thermometer, limewater, test tube, glass tube, large-mouthed bottle, pneumatic trough.

Directions.—*A. Temperature.* Breathe on the bulb of the thermometer and determine the temperature of the expired air. Place the bulb under the tongue and determine the body

temperature. How does the temperature of the expired air compare with that of the body, or blood temperature? Test this on several successive days and note whether the temperature varies with the external temperature or is constant.

B. Composition. Breathe on a piece of glass. What collects on the surface? Does expired air contain more or less moisture than inspired air?

Fill the test tube half full of limewater and blow the breath gently through it by means of the glass tube. What change takes place in the limewater? What does this indicate? (See Ex. V.)

Fill the bottle with expired air by the method of Ex. II. Turn the bottle mouth upward and introduce a lighted match into it. Does the match continue to burn? What does this indicate? (Air expired in ordinary breathing has lost about one-fourth of the oxygen contained in the air inspired.)

EXCRETION

LXI.—STUDY OF A LAMB'S KIDNEY (OPTIONAL).

Apparatus.—A fresh lamb's kidney with its capsule of fat, scalpel.

Directions.—Carefully remove the outer layer of fat and the membranous inner capsule. What is the function of this material? (A dissection of the rat makes a good demonstration of the location of the kidneys and their relation to ureter and bladder.) Cut the kidney lengthwise so as to split the ureter where it emerges from the concave side. On the cut surface make out the pale inner striated *medulla* and its *pyramids of Malpighi*, the outer *cortex*, and the *intermediate layer* between the two. Note also the enlarged upper end, or *pelvis*, of the ureter; the cavity, or *sinus*, into which it opens; and the tubes, or *calices*, between the projecting pyramids. Note also the entrance of the renal artery into the kidney, and the renal vein, just above the ureter. From the accompanying Fig. 44 make

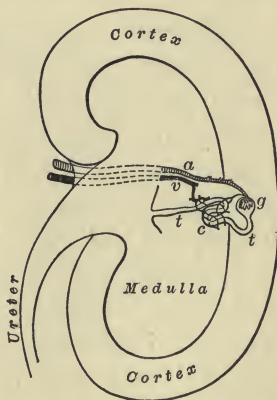


FIG. 44.—Diagram of a Longitudinal Section of a Kidney: *a*, renal artery; *c*, capillaries; *g*, glomerulus; *t*, uriniferous tubule; *v*, renal vein.

out the parts which act in removing the waste. (The artery brings in the blood, which gives up its waste in the glomerulus. This waste is collected by the tubule and emptied by it into the ureter. The capillaries collect the blood which has been cleared of its waste, and return it to the vein.)

NOTE.—Prepared sections of injected and stained cortex may be shown and the following parts demonstrated: Malpighian bodies, uriniferous tubules, and capillaries.

LXII.—STUDY OF THE SKIN.

Apparatus.—Prepared slide of epidermis (that from the sole of the foot preferred, from its thickness), a vertical section of a hair, compound microscope, needle, scissors.

Directions.—*A. Surface Study of the Skin Layers.* Sterilize a needle by holding it in a flame a moment. Run it carefully under the thin outer layer of skin at the base of the thumb. (This layer is called the cuticle or *epidermis*.) Does the wound cause any pain? Are there any nerves in this layer? Does the wound bleed? Does the epidermis contain any blood vessels? With the needle tear off a little of this epidermis. What is its color? consistency? Where is it thickest on the hand? Why? Where else on the body do you find similar thickening?

What is the color of the skin layer (*dermis*) under this epidermis? Prick it with the needle. Is it sensitive? Does it contain blood vessels? Examine its surface and note that it is ridged. A magnifier will show that these ridges are made up of a series of points, or *papillæ*. (Each papilla marks the end of a nerve of touch. These nerve endings are called on that account tactile organs; see Fig. 45.)

Pick up a little of the skin between the fingers. Is it attached to the underlying muscles? About how thick is it on the back of the hand? on the base of the thumb?

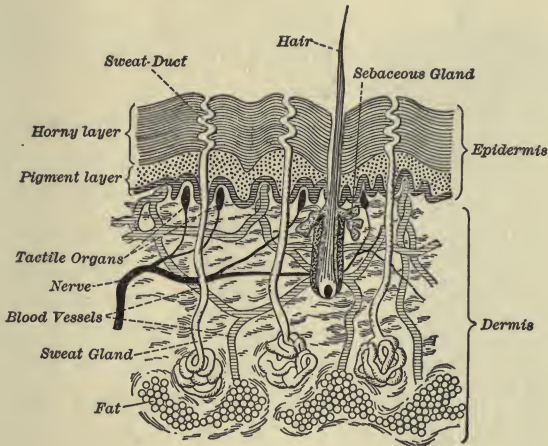


FIG. 45.—Diagram of Skin Section.

B. Microscopic Study of a Section. Study the prepared slide, under the high power. Note the layer character of the epidermis, the papillæ with their blood vessels, the coiled *sweat glands* and their ducts (thick sections show these best). Sketch and label all parts of your drawing as in the diagram.

Compare the action of the sweat glands with that of the tubuli uriniferæ (uriniferous tubules) of the cortex of the kidneys. When do we perspire most? Why does exercise increase the amount? What is one function of the skin?

C. Study of Skin Modifications. (a) Hairs. Note the

location of hair on the head. What is its function? Examine one of the hairs on the back of the hand. Cut it. Is it sensitive? Pull it. Where is the sensitive portion located? Where is the seat of growth? What part of the skin is it most like?

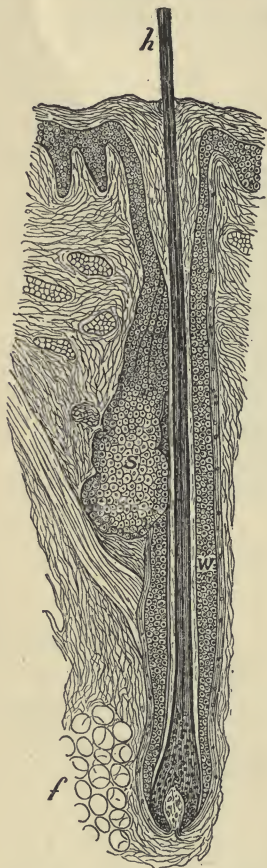


FIG. 46.—Hair-follicle in Longitudinal Section: *h*, hair shaft, showing its medulla or core; *s*, sebaceous gland; *w*, sheath of skin; *f*, fatty tissue. (At the base of the hair is seen the papilla that forms it.)

Study the slide showing a vertical section of a hair, under the low power of the microscope. Note that the hair is imbedded at the base in a skin *follicle*, and grows from a skin *papilla* at the bottom of this follicle. Note also the *sebaceous* or *oil glands* that serve to coat the hair with oil.

(b) Nails. Make a drawing of your finger nail, showing all areas. What parts are attached to the skin? Why is the part under the nail called the “quick”? What is one function of the nail? Cut it. Is it sensitive? Pull it. Where is its sensitive part located? How does it compare with the hair in this respect? Cut a nick in it and examine it from day to day. Does it change position? Where does the growth of the nail occur?

Tabulate all the functions of hairs, nails, and skin that you have learned.

NERVOUS SYSTEM

LXIII.—DISSECTION OF SHEEP'S BRAIN.

Apparatus.—Sheep's head, bone forceps, hammer, scalpel, needle, forceps, 50% alcohol.

Directions.—*A. To Remove Brain from Skull.* Strike the top of the skull with the hammer so as to crack the bone, but not to force it into the brain; and then carefully remove the pieces with the bone forceps. Be careful not to injure the underlying membrane (*dura mater*) which lines the skull and covers the brain. After the top of the skull is removed slit this *dura mater* around the edge, and remove it, exposing the brain. Note that over the surface of the brain is another membrane, the *pia mater*. Now carefully lift the brain from the floor of the skull, beginning at the front. Notice that it is bound by nerves and portions of the *dura mater*. Cut these nerves, leaving as long ends as possible, and do not cut off the olfactory lobes which are on the under side of the brain. Place the brain in 50% alcohol to harden, for several days.¹

B. The Coverings of the Brain. Tear off a little of the *dura mater* with the forceps. Does it tear easily? Are both sides of it smooth? Where are its blood vessels? What do they feed?

Pick up a little of the *pia mater* (brain cover) with the

¹ Preserve the skull, with eyes, for use in Ex. LXX.

needle point. Is it thicker or thinner than the dura mater? Where are its blood vessels? What do they feed? What are the functions of the three coverings of the brain?

C. The External Parts of the Brain. Examine the top of

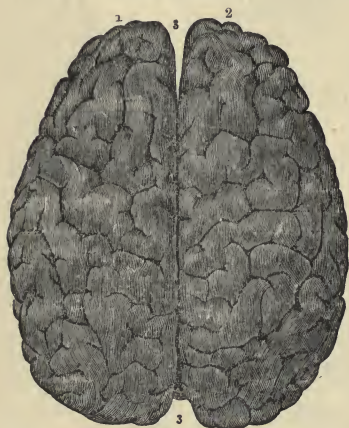


FIG. 47.—Upper Surface of Brain (Human): 1, 2, two halves, or hemispheres, of cerebrum; 3, 3, longitudinal fissure.

the brain. Note the two convoluted hemispheres into which the fore brain (*cerebrum*) is divided by a fissure (the *longitudinal fissure*). Back of this appears the wrinkled surface of the hind brain (*cerebellum*). Is this divided?

Turn the brain over and examine the lower surface. Note the *olfactory lobes* on the front part of the hemispheres. What is their function? Back of these, locate

the *optic nerves* and note how they cross to form a *chiasma*, so that the right eye is controlled by the left hemisphere, and vice versa. Just back of this may be seen the *pons*, or bridge, that connects the two sides of the cerebellum, and, coming out in front of it on each side, the stalks (*crura cerebri*) which spread out into the two hemispheres of the cerebrum. Note that the stalks are the forward projections of a conical spinal bulb, which comes between the cerebellum and the pons, and is continued backward into the spinal cord. This bulb is a part of the hind brain, and is called the *medulla*. All along the under side of the brain are located the cranial nerves, occurring in pairs. Beginning at the front locate the pairs named in the following table:

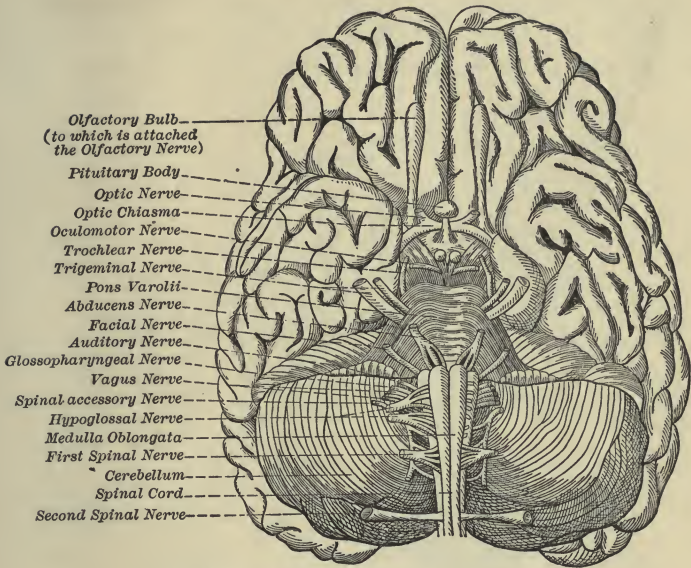


FIG. 48.—Under Surface of Brain (Human).

	NAME.	FUNCTION.
1st pair	Olfactory	Smell—Sensory
2d pair	Optic	Sight—Sensory
3d pair	Oculomotor	Eye Muscles—Motor
4th pair	Trochlear	Eye Muscles—Motor
5th pair	Trigeminal	Facial—Sensory and Motor
6th pair	Abducens	Eye Muscles—Motor
7th pair	Facial	Motor
8th pair	Auditory	Hearing—Sensory
9th pair	Glossopharyngeal	Tongue and throat—Sensory and Motor
10th pair	Vagus	Thorax and abdomen—Sensory and Motor
11th pair	Spinal Accessory	Sensory and Motor
12th pair	Hypoglossal	Tongue—Motor

D. Vertical Section (right side). Cut the brain through lengthwise, parallel to the line of the longitudinal fissure, but one-sixteenth of an inch to the left of this line in order not to cut the septum. Examine the right side. Note the

white, fibrous body (*corpus callosum*) which unites the two hemispheres. In the front part of this are seen the thin membranes (*septum lucidum*) which inclose between them

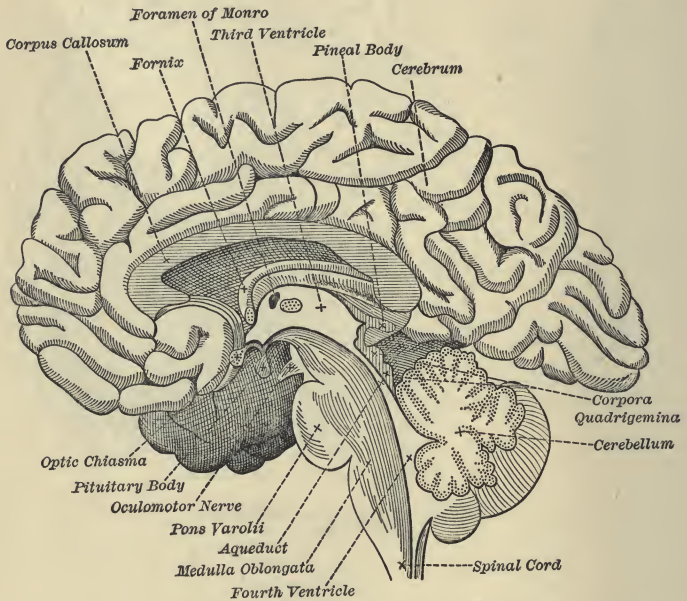


FIG. 49.—Section of Brain.

a portion actually outside the brain, but often called the "fifth" ventricle. Below this is the *fornix*, which forms the roof of a true brain cavity—the *third ventricle*—whose sides are the *optic thalami*. This ventricle projects forward into a funnel called the *infundibulum*. In the center of this ventricle is a round body (the *median commissure*) which was cut through by the section. In front of this is a small aperture (the *foramen of Monro*) that connects this cavity with a cavity in the right hemisphere. The floor of the

third ventricle is formed by the crura cerebri, which extend backward, between the pons and cerebellum, into the spinal bulb or medulla, and this, in turn, backward into the spinal cord. At the back of the third ventricle note that a tube or canal (*aqueduct*) connects it with a much smaller cavity (the *fourth ventricle*) just under the cerebellum. Four little bodies (the *corpora quadrigemina*) form the roof of this tube between the fornix and cerebellum. Note the tree-like internal structure of the cerebellum. What causes its wrinkled surface?

Note the gray and the white matter that make up the cerebrum. Where is the gray matter located? the white?

NOTE.—The first ventricle in the olfactory lobes and the lateral ventricle may be shown by suitable sections, if desired, and the relation of these may be brought out by the aid of diagrams of the simple brain structure.

LXIV.—DISSECTION OF SPINAL CORD.

Apparatus.—Thin section of cervical portion of spinal cord, glycerine, slides, cover glass, compound microscope.

Directions.—(Prepare sections by placing a piece of the cervical spinal cord for three or four weeks in Müller's fluid [$2\frac{1}{3}$ parts of potassium bichromate, 1 part of sodium sulphate, 100 parts of water]. Then wash it with water and place it in 30% alcohol for a few days. Then transfer it to 95% alcohol. Cut a thin cross section and mount it in glycerine. Cover it with a cover glass).

Examine under the low power. Note the outer covering of *pia mater*. Note the distribution of the gray and white matter. Sketch it. Is it the same as in the brain? Note

the division into two parts by a deep anterior, and shallow posterior fissure. Note also two fissures in each half (anterior and posterior lateral) through which the central gray mass reaches the surface. The gray masses in each half of

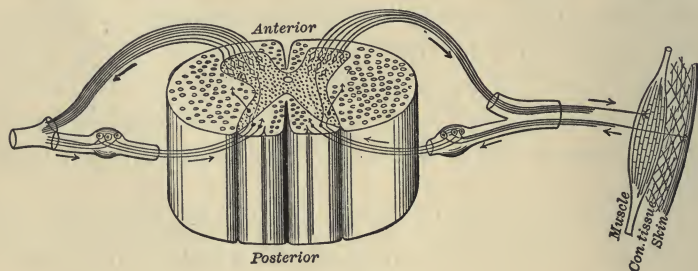


FIG. 50.

the cord may be seen to be united by a commissure that incloses the central or neural canal. Note the cellular character of the gray matter. The gray matter that forms the posterior horns forms the core of spinal nerves of the sort called afferent. The anterior horns form the core of efferent nerves. (Afferent nerves carry messages to the cord, efferent away from it.) The white matter covers these and they unite outside in a common spinal nerve. (See Fig. 50.) For structure of a neuron, see Ex. XXVIII.

SPECIAL SENSES

LXV.—NERVE ACTION.

Apparatus.—A stop watch, pencil, paper.

Directions.—Let the teacher write a vowel on a piece of paper which he shall keep covered. Arrange the class in a circle. Station a boy beside the teacher with a stop watch. Proceed as follows: The teacher shows the vowel to the pupil on his right, who whispers it to the pupil on his right as quickly as possible, and so on around the circle to the teacher again. All this as rapidly as possible. Let the boy with the watch release the stop at the second when the teacher exposes the letter to the pupil on his right, and stop it again when the last pupil repeats the letter to the teacher. Divide the time elapsed by the number of pupils. The result will represent the average reaction time of each pupil. Change the arrangement of the pupils and note whether the time varies. What muscular action does each pupil perform in receiving and transmitting the sound? What sensory nerves are employed? what motor nerves?

NOTE.—In order to bring out various sensation reactions this experiment may be varied in many ways which will suggest themselves to the teacher.

LXVI.—CUTANEOUS SENSATIONS.

Apparatus.—A pair of metal compasses, toothpicks, a dish of boiling water, a dish of ice water, pen and ink.

Directions.—One pupil should operate, another acting as subject. The subject should be blindfolded.

A. Touch. Sharpen the ends of the toothpicks and tie one to each arm of the compass. What is the least distance apart at which the two points may be held and felt as two points, when applied to the tips of the fingers? the tip of the tongue? back of the hand? forearm? back of the neck? Record the results. Are all parts of the body equally sensitive to touch? Which parts are most sensitive?

B. Temperature. Dip a metal point of the compasses in cold water and move it lightly over the back of the hand. Does it feel equally cold to all parts of the skin? Mark with an ink dot those points where the sensation is most acute. Now dip the metal point in the hot water and move it over the same area. Locate, as before, the spots where sensation is most acute. Do the hot and cold spots coincide? What do you conclude about the temperature sensation power of the skin? Is it a general or a localized sensitive power? Test other areas of the body in the same way. Are the temperature spots equally numerous in all parts? Where are they most numerous? least numerous?

LXVII.—STUDY OF THE TONGUE.

Directions.—Protrude the tongue as far as possible and with the aid of a mirror examine its surface. Note the raised points (the *papillæ*) on the surface. Observe that they are of three forms: long and slender (*filiform*), mush-

room-topped (*fungiform*), and large and wartlike (*circumvallate*). Draw an outline of the tongue and locate on it the regions where these different forms are to be found.

LXVIII.—SENSATIONS OF TASTE AND SMELL.

Apparatus.—Onion, sugar, salt, vinegar, dilute ammonia, quinine.

Directions.—*A. Location of Taste.* Wipe the tongue dry and place on its tip a little dry sugar. Has it any taste? Let it dissolve. Has it any taste now? Repeat, placing the sugar at the back of the tongue. Is its sweetness more or less prominent? Repeat again, using quinine, vinegar, and salt successively. Where are the sensations of bitterness, sourness, and saltiness most prominent?

B. Taste and Odor. Examine the various substances named under "Apparatus." Which have taste? odor? Place each of these substances on the tongue of a pupil who has been previously blindfolded, and who is holding his nose tightly. Record the substances recognized by taste alone. Repeat, leaving the nose free but retaining the blindfold. Record those substances recognized by smell alone; by taste and smell combined.

LXIX.—HEARING; LAWS OF SOUND (OPTIONAL).

Apparatus.—Stretched wire, bridge to shorten length.

Directions.—*A.* Strike the wire. Do you get any sound? What is the wire doing? All sound depends upon vibration: test several sounding bodies to verify this statement.

B. Move the bridge to the middle point of the wire and strike again. Is the pitch higher or lower? Does a short

string vibrate faster or slower than a long one? What effect has rate of vibration on the pitch of a sound?

C. Strike the wire gently. Note the distance at which the sound can be heard. Strike harder. Is the tone louder or softer? Can it be heard at a farther distance? Does it vary in pitch? What effect on sound does extent (amplitude) of vibration have?

D. Stand at the point where you can just hear the ticking of a watch. Now make a conical tube of paper and insert the small end in the ear. Point the large end toward the watch. Can you hear it any better now? What part of the ear serves a purpose similar to that of the tube?

LXX.—VISION; DISSECTION OF SHEEP'S EYE.

Apparatus.—Sheep's skull with eyes in socket (the skull used in Ex. LXIII will serve for this purpose), scalpel, scissors, bone forceps, evaporating dish.

Directions.—Cut away, with the bone forceps, the bones that inclose the eye, so that it may be seen in position from the side.

A. *Muscles.* Notice that the motion of the eyeball is controlled by six muscular bands. Locate the attachment of four of these bands on the top (*superior rectus*), bottom (*inferior rectus*), side near nose (*internal rectus*), and side farthest from nose (*external rectus*). Note that these extend directly backward to the end of the socket and have their origin there. What motions do these muscles give to the eyeball? Now locate on the top of the eyeball the attachment of a transverse band of muscle (the *superior oblique*) and follow its course, through a tendon pulley, to its origin at the back of the socket. In what direction

does its contraction take place? What motion does it give to the eye? On the under side of the eye locate another transverse muscle (the *inferior oblique*). Where is its origin? Has it a pulley?

B. The Externals of the Eye. Cut the muscle bands and trim away a white membrane (the *conjunctiva*, a continuation of the lining of the eyelid) in the front of the eye. Note that the eye is still attached to the socket by a cord, just below and outside the center of its rear surface. This is the *optic nerve*, which enters the eye here from the brain. Pull the eye out of the socket and cut this cord. Now examine the outside of the eyeball. Note that it is covered with a firm white coat (the *sclerotic*) except in the front, where there is a clear layer, the *cornea*, usually dulled in death.

C. The Internals of the Eye. Hold the eye with the cornea uppermost, and remove this with the scalpel by cutting horizontally around its edge. The liquid back of this layer is the *aqueous humor*. Directly back of the cornea appears a circular muscular curtain—colored in the human eye—called the *iris*, and in its center a hole—the *pupil*. What conclusions do you draw as to the functions of this iris from comparing the size of the pupil of your own eye, when looking at a bright light, with its size when in a dimly lighted room? Is its action voluntary?

Now lay the eye upon its side in the evaporating dish and cover it with water. With the scalpel cut a section through the entire eye, splitting the optic nerve (see Fig. 51). Observe the following parts: just back of the iris the convex *crystalline lens* and its capsule; the muscles that control the shape of the lens—the *ciliary muscles*—and their ligamentous attachment (*suspensory ligament*); on the inside of the layers that form the walls of the eye, at the edge of

the lens, a black, ridged membrane (the *ciliary process*); the jelly-like mass that fills the body of the eye (*vitreous humor*); the three layers of the wall of the eyeball—outer (*sclerotic*), middle (*choroid*), inner (*retina*).

Note that the optic nerve pierces the two outer coats and spreads out to form the retina. Remove the vitreous humor and notice the soft, whitish retina. Tear this out with the

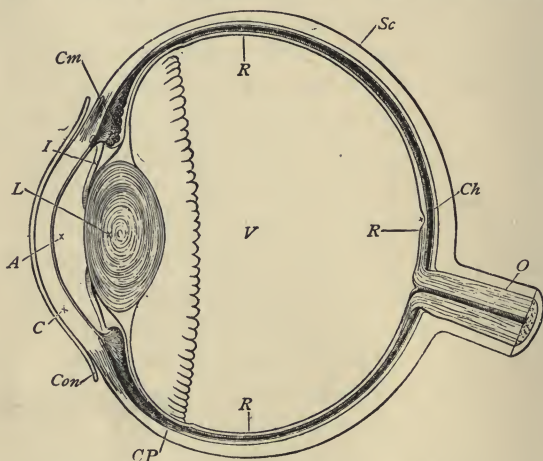


FIG. 51.—Cross Section of the Eye: *Con*, conjunctiva; *Sc*, sclerotic; *C*, cornea; *A*, aqueous humor; *I*, iris; *L*, crystalline lens; *Cm*, ciliary muscles and ligament; *CP*, ciliary process; *V*, vitreous humor; *Ch*, choroid; *R*, retina; *O*, optic nerve.

forceps and note its consistency and thickness. Under this observe the color and luster of the choroid coat. When this is torn out, the interlacing blood vessels are seen passing from one layer to the other.

LXXI.—ACTION OF THE EYE.

Apparatus.—Model of eye.

Directions.—Construct a model of the eye as follows:

Obtain a wooden box eighteen or twenty inches long and about eight inches wide and deep. Leaving one side open, paint the inside of the box black. Around the open side tack a piece of black cloth large enough to cover the head of the observer and shut out the light from the interior of the box. At one end of the box cut a hole one inch in diameter. Cut several black cardboard disks to fit this aperture, and perforate their centers with holes varying from one-sixteenth to one-half inch in diameter. Mount a convex lens in a movable holder which can be moved forward and backward on the floor of the box, and which will bring the center of the lens opposite the center of the hole. Mount

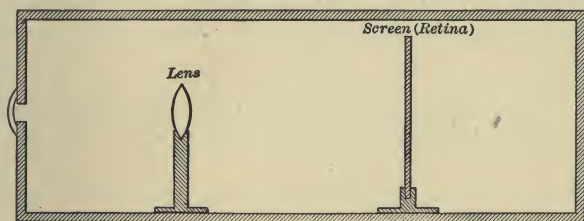


FIG. 52.

a piece of ground glass in the same way to serve as a screen. Arrange all parts as in Fig. 52.

The cardboard disks will then correspond to the iris with its pupil; the walls of the box to the sclerotic; the black paint to the choroid (what is its function?); the lens to the crystalline lens, and the screen to the retina. A watch glass placed on the aperture would resemble the cornea.

A. Action of Parts. Darken the room and place a lighted candle at a distance of three feet from the aperture. Place in the aperture the disk with one-quarter inch perforation. Cover head with cloth and place screen at the rear of the

box. Now move the lens back and forth until there appears on the screen a sharp image of the candle flame. Is it right side up? What is the function of the lens? Mark position of lens and screen. Move the candle three feet farther away. Does the image remain on the screen? Keeping the lens fixed, move the screen forward in the box until the distinct image appears again. Return the screen to its original position and, by moving the lens, cause the same result—an image on the screen. Which is adjustable in the eye—the screen (retina) or lens? How is the lens adjusted in the eye? (This adjustment of the lens to the distance of objects is called *accommodation*.) Change the disks in the aperture, using first larger and then smaller openings. Which gives the brightest image? What is the function of the iris?

NOTE.—By using external lenses as “spectacles,” short- and long-sightedness can be corrected and illustrated.

BACTERIA

LXXII.—STUDY OF BACTERIA.

Apparatus.—Hay, milk, meat, test tubes with corks to fit, slides, cover glasses, compound microscope, sterilized absorbent cotton, methyl green, corrosive sublimate.

Directions.—*A.* Prepare three test tubes as follows: Put in one some pieces of chopped hay and cover with water; in the second some milk; in the third some meat, covered with water. Let these stand uncovered, in a warm place, for several days. Describe the changes that take place in the appearance and odor of the contents. Note the scum that appears on the surface. Mount some of this scum on a slide and cover with a cover glass. Examine with the high power of the microscope. Note the masses of moving forms (*bacteria*) that make up this scum. Sketch the shape of some of these bodies. (If a drop of methyl green be run under the cover glass, the structure will be clearer.)

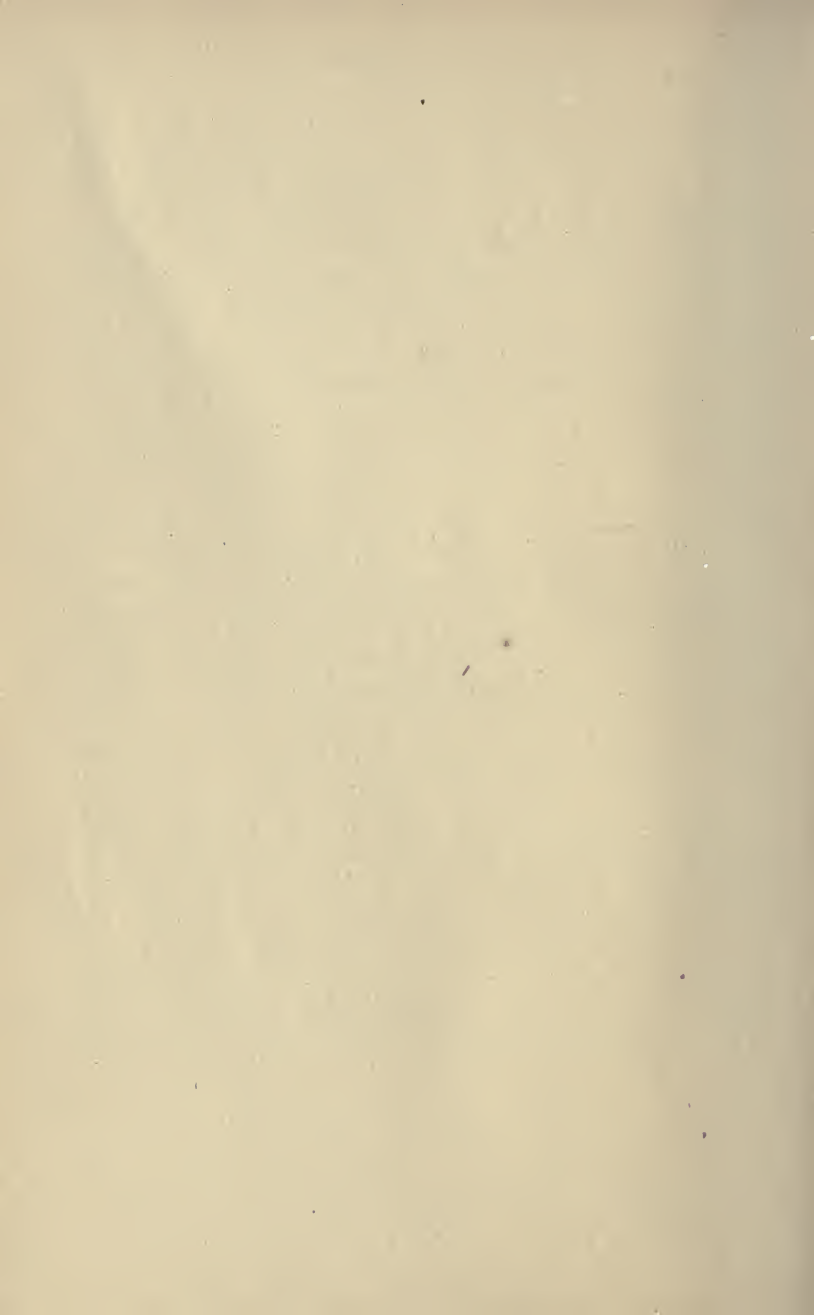
B. Prepare four tubes as follows: Fill each tube with milk. Pack one in ice; leave the top open and label it No. 1. Boil the contents of the second, stopper with a cork that has been boiled in water, and leave in a warm place; label this No. 2. Boil the contents of the third one and stopper with sterilized absorbent cotton; label this No. 3. Place No. 3 with No. 2. Place the fourth, labelled No. 4, in a warm place, uncorked. After several days examine the

four tubes. In which is the milk still fresh? What is the effect of heat on bacteria? of cold?

Remove the stopper from No. 2 and the ice from No. 1, and place with No. 3 for several days longer. After that time examine the tubes. In which is the milk still fresh? From these experiments, what are your conclusions as to the source of bacteria? Is air that is freed from bacteria by being strained through cotton, a cause of decay?

C. Prepare two tubes as follows: Boil some milk and fill two tubes that have been previously boiled in water. Stopper with sterilized cotton and allow the tubes to cool to room temperature. Now introduce into one tube some scum from the hay infusion and restopper quickly. Set it in a warm place. Prepare a two per cent solution of corrosive sublimate. Mix a teaspoonful of this with some scum from the hay infusion and let it stand for ten minutes. Then introduce this into the second tube and stopper again quickly with the cotton. Place it with the other tube. Examine after a day. In which tube has the milk decomposed? What must the corrosive sublimate have done to the bacteria? What substance may be used to kill bacteria in place of heat? Killing bacteria by heat is called sterilizing; chemical substances that perform the same action are called *antiseptics*. What are some of the common antiseptics?

Summarize your results under the following heads: (1) conditions favorable to bacteria growth; (2) conditions unfavorable to bacteria growth; (3) methods of killing bacteria.



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